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ABSTRACT

Here I summarize and expand an innovative paradigm that updates Darwin and the Modern Synthesis with a mechanism that explains how rapid evolutionary change in multi-cellular organisms can be initiated and implemented. This new theory hinges on the premise that thyroid hormone (TH, T_3 and/or T_4) plays a pivotal role in speciation and adaptation. In most animals TH can be absorbed from ingested food as if it were a vitamin but in vertebrates, it is also manufactured (like a classic hormone) in a distinctly rhythmic fashion. I argue that the rhythmic secretion of manufactured TH (or its analogue in non-vertebrates, including plants and insects) is an under-appreciated feature of TH metabolism that has fundamental biological and evolutionary significance. I contend that this hormonal mechanism can not only explain domestication as a natural speciation process (e.g. the transformation of wolves into dogs) but change over time in virtually all multi-cellular organisms, including our human ancestors. In part, this mechanism works through TH regulation of many developmental genes in a dose- and time-dependent manner. I suggest that during colonization of new habitats, small founder populations possess a non-random subset of ancestral TH rhythms phenotypes and that this loss of hormonal variation initiates rapid heterochronic changes via shifts in developmental gene function. Increases in quantity of ingested TH, due to habit-associated changes in food sources, can compound these effects to generate the macro-evolutionary modifications seen in higher-level taxa. This integrative evolutionary theory takes into account all we have learned in recent decades about the unique attributes of TH (particularly its critical influence on development genes and other hormones) and the fundamental nature of all biological rhythms, all of which have profound implications for evolutionary biology. Most importantly, this unifying paradigm offers a long-overdue addendum to the exclusively genocentric view mandated by the Modern Synthesis: it not only addresses the so-called “species problem” but makes evolution uniquely personal. The concept thus provides a testable theoretic framework for a 21st century update in evolutionary biology and medical science.

KEY WORDS: thyroid hormone, heterochrony, evolutionary theory, Modern Synthesis, domestication, speciation, macroevolution, bipedalism, hypothyroidism, iodine.

INTRODUCTION

It has been more than 60 years since the so-called "Modern Evolutionary Synthesis" updated Darwin's (1859) evolutionary principles with the addition of a genetic paradigm (Mayr, 1942). However, evolutionary biologists have not, to date, been able to suggest an explicit biological mechanism that accounts for precisedly how most species and higher level taxa originated (the so-called "species problem"). Speciation is the most fundamental of all evolutionary processes and yet our current genocentric understanding of speciation does not really take into account recent knowledge of the variable time frame of speciation, the conserved nature of many developmental genes, the critical role that hormones play as regulators and coordinators of gene function, nor the profound effects that biological rhythms exert on critical life history traits. Although I am not the first to say so, I contend that the Modern Synthesis is in serious need of a revision that explicitly incorporates this new knowledge.

There is growing evidence, for example, that much more often than expected, speciation can be very rapid indeed, not always slow and gradual as both Darwin and proponents of the Modern Synthesis have insisted (Hendry *et al*., 2000; Pagel, Venditti & Meade, 2006; Rüber, Van Tassell & Zardoya, 2003; Sullivan *et al*., 2002; West-Eberhard, 2003, 2005; Wiens, *et al*., 2006; Vrba, 2005). We have also known for some time that there are not nearly enough discrete genes to account for all of the changes that occur during speciation in multi-cellular organisms (e.g. Hall, 2003; Hlusko, 2004; Pennisi, 2007). Epigenetic modifications are known to change gene function from one generation to another without genetic mutation (e.g. Jirtle & Weidman, 2007) and yet, our understanding of evolutionary change is still firmly tied to the concept that genetic mutation is paramount (e.g. Futuyama, 2005; Hoekstra & Coyne, 2007). Above all, there is a wealth of new information regarding the multi-faceted role that thyroid hormone plays in the regulation of system-wide gene expression and cell signalling in the coordination of growth, development, stress response, reproduction, and metabolism in a range of organisms (Heyland *et al*., 2006; Hulbert, 2000; Liu & Brent, 2005). Finally, we are coming to appreciate just how fundamental circadian and hormonal rhythms are to basic body functions and gene expression (e.g. Ptitsyn, Zvonic & Gimble, 2007). Despite this new knowledge, however, a statement made by Ernst Mayr (1988, 208) almost twenty years ago is unfortunately true today: "in spite of all the advances of genetics, we are still almost entirely ignorant as to what happens genetically during speciation."

The emerging field of evolutionary developmental biology, "evo-devo," has huge potential for elucidating how and why organisms change over time but still lacks a unifying theoretic framework (e.g. Hall, 2003; Müller & Newman, 2005; Salazar-Ciudad, 2006). While heterochrony (changes in developmental rates and/or timing) has been successfully argued as the most likely means of rapid evolutionary change in multiple traits ─ with heterochronic change identified in virtually all lineages of multi-cellular organisms, including vertebrates, invertebrates and plants ─ many evo-devo researchers have taken a purely genetical approach to providing a mechanism to explain implementation of heterochronic change. Evo-devo proponents are thus no further ahead than evolutionary geneticists (e.g. Hoekstra & Coyne, 2007). However, it has become apparent that the mathematical population genetic models that served well for explaining some cases of microevolutionary change and adaptation (especially in fruit flies), which many assumed could be extrapolated across the board to explain all phenomena in all taxa (e.g. Gavrilets 2003), are simply not adequate for addressing heterochrony.

The interactions between genotypes and phenotypes that affect developmental processes in heterochronic fashion are not linear, one to one relationships but complex webs of interdependence that affect morphology, physiology, reproduction and behaviour. While many biologists are cognizant of this fact, no one has yet supplied a comprehensive paradigm that successfully takes these complex relationships into account (e.g. Goldenfeld & Woese, 2007; Hansen, 2006; Hendry, 2007; Poelwijk *et al*., 2007; Salazar-Ciudad, 2006). Although some have attempted to characterize these inter-relationships in ecological terms, no precise mechanism for implementation has yet been suggested (e.g. Badyaev, 2005; Breuker, Debat & Klingenberg, 2006; Estes & Arnold, 2007; Gould, 2002; West-Eberhard, 2003). Neither has anyone, to my knowledge, proposed a specific link between gene function, individual variation and environmental change, although it is clear that establishing such a link is critical for explaining why so many unrelated lineages, over vast expanses of evolutionary time, transform in similar fashion in response to shifing climate (e.g. Vrba, 2005).

Here I offer a novel, interdisciplinary perspective on the issue, starting─as Darwin (1868) did─with domestic animals, in part because they include some of the best understood examples of heterochrony. Although domesticates have traditionally been defined as products of human innovation (e.g. Gepts, 2005), this view is now regarded as untenable, anthropological dogma (e.g. Dobney & Larson, 2006; Crockford, 2004). In its place, I provide a model with a testable

hypothesis to explain the initial transformation from wild to domestic form (which I call "protodomestication") as natural heterochronic speciation; I then apply the concept to all vertebrates (Crockford, 2002, 2004, 2006).

Integral to this model is evidence that thyroid hormones (THs, a collective term for T_3, triiodothyronine and/or T_4, thyroxine) are essential regulators of all traits that undergo change during protodomestication, strongly implicating THs as pivotal to the process. THs have wide-ranging control over genetic and cellular function: many, many genes that govern fundamental development and life history processes respond to THs in a time- and dose-dependent manner. While other hormones have critical and often multi-faceted roles, THs alone are essential for day to day maintenance functions of adults in an environmentally-responsive manner as well as growth and development of embryos from the moment of conception onward.

All hormones, including TH, are known to be secreted in a distinctly pulsatile fashion (e.g. Chadwick & Goode, 2000; Lucke *et al*., 1977). Although some researchers consider TH rhythms to be unremarkable phenomena, this assumption has not been tested. I contend that strong circumstantial evidence exists for my claim that TH rhythms, in conjunction with better-known circadian rhythms, have enormous evolutionary and biological significance (e.g. Ptisyn *et al*., 2007). I propose that the rhythmic secretion of TH may be the elusive biological mechanism responsible for evolutionary transformation we have sought for so long. Rhythmic secretion of TH, which appears to be the hormonal pacemaker critical to coordinated function of other hormones, can explain rapid heterochronic change and changing gene function in an environmentally-responsive manner. Surprisingly, while a hormonal basis for heterochronic speciation has long been suspected, a pivotal role for THs in vertebrate evolution has not been suggested before now. As a consequence, virtually no research effort has been expended comparing the phenomenon of TH rhythms within and between species.

The concept that timely availability of TH (hereafter, "TH rhythms") play a pivotal role in vertebrate evolution becomes a novel paradigm with the realization that invertebrates and plants not only have analogous hormonal control systems that operate in similar fashion, but heterochronic change is also a dominant pattern in their evolutionary histories. I contend, therefore, that THs, alone or in conjunction with their analogues in other kingdoms (Heyland & Moroz, 2005), have a pivotal role in the initiation and implementation of evolutionary change in many multi-cellular organisms. While this paradigm challenges one of the accepted tenets of neo-

Darwinian theory—that evolution is due almost exclusively to the gradual accumulation of random genetic mutations and as a consequence is imperceptively slow—it more accurately reflects and predicts the complex nature of inter- and intra-specific relationships we are now able to discern from phylogenetic analysis of closely related species (e.g. Bennetts *et al*., 1999; Grant, Grant & Abzhanov, 2006; Ryan *et al*., 2007; Smith *et al*., 2007; Talbot & Sheilds, 1996).

This new paradigm also has profound implications for human health, since it is apparent from recent and on-going medical studies (e.g. Boas *et al.,* 2006; Lacasse & Leo, 2005; Laurberg, 2006; Lemkine *et al*., 2005; Vaidya *et al.*, 2007; Watt *et al*., 2006) that we must have a better understanding of how variable TH levels regulate our bodies as we grow, reproduce and age if we are to adequately prevent, diagnose and treat our most chronic and debilitating ailments (including cardiovascular disorders, depression, infertility, birth defects, mental retardation, obesity and the TH-disruptive effects of chemical contaminants). As a consequence of these impacts on human health, this new concept makes the need to understand evolution profoundly personal, a result which should assist biologists enormously in their endeavour to make citizens understand the importance of evolution to society.

In the interest of brevity and because this is meant to be an update as well as a summary, I have (with a few exceptions) not repeated references that appear in previous publications (especially Crockford 2004, 2006) but include primarily new ones.

REIGNING PARADIGM

The Modern Evolutionary Synthesis, conceived in the early 1940's, merged the doctrines of population genetics with Darwinian principles (e.g. Futyama, 2005; Mayr, 1942, 2001). By adding the knowledge that genes control inherited characteristics and change over time via random mutation, Darwin's concept of natural selection became widely accepted as the major driving force in evolution. However, the population genetic paradigm on which the Synthesis is built depends heavily on the "one gene, one trait" concept carried over from Mendel: it assumes that traits subject to natural selection—the phenotypic variation contained within a population that is necessary for evolution to occur—is due to multiple alleles of trait-regulating genes. Although the fact that multiple traits change during speciation is acknowledged, in most studies each trait is assumed to be under separate genetic control and thus altered independently via selection for different adaptive purposes. The reigning paradigm also insists that because speciation is

dependant on the gradual accumulation of small genetic changes, speciation is virtually always imperceptively slow; in addition, however, more profound changes (i.e. macroevolution) are considered to be simply more of the same spread over longer periods of time (e.g. Hlodan, 2007; Erwin, 2000).

With ever-increasing evidence that these assumptions are largely incorrect, at least for many multi-cellular organisms, weak spots within the Modern Synthesis that were recognized decades ago are becoming more problematic with time, not less. Stephen Jay Gould (1980:120) proclaimed more than 25 years ago that "if Mayr's characterization of the synthetic theory is accurate, then that theory, as a general proposition, is effectively dead, despite its persistence as text-book orthodoxy." It is now the 21st century and while the synthetic theory has not died, neither has it been significantly updated. Instead, the operating principle seems to be that if genes are scrutinized ever more closely (e.g. Hansen, 2006; Pennisi, 2007; Whitehead & Crawford, 2006), ensuing discoveries will make the contradictions of the Synthesis disappear.

Traditionally, small mutations within regulatory genes that operate during embryonic development were proposed as the most probable means to attain large phenotypic change without substantial genetic change but is it now clear that most regulatory genes are remarkably conserved (e.g. Hlodan, 2007; Prud'homme, Gompel & Carroll, 2007). One new research approach that by-passes this problem uses a genocentric version of the original "evo-devo" concept of epigenesis (e.g. Müller, 1990; Müller & Newman, 2005). The premise of this epigenetic model is that attachment of methyl groups to certain portions of genes (or similar such modifications) can profoundly affect gene activity without actual mutational change (e.g. Jirtle & Weidman, 2007; Murrell, Rakyan & Beck, 2005). Results of preliminary work indicate that epigenetic effects may have evolutionary significance (e.g. Weaver *et al*., 2004), a premise I discuss later in this essay. As a consequence, studies that examine differences in gene function via gene expression patterns are becoming increasingly common, although evidence is also mounting that genes themselves are not quite what we once believed (e.g. Gerstein *et al*., 2007; Gingeras, 2007; Storey *et al*., 2007; Whitehead & Crawford, 2006). However, while an epigenetic approach eliminates the necessity of finding genetic mutations that explain differences between taxa, it does nothing to resolve the real conundrums of the Synthesis (e.g. Müller & Newman, 2005). Invoking epigenetics does not explain how rapid speciation begins, how and

why it proceeds, or why there are such recurring suites of changes evident in vastly different lineages of plants and animals over extended periods of geological time.

In all multi-cellular organisms, the life history traits critical for adaptation to new environments are complex linked characteristics regulated as much by hormones and environmental factors as genes (e.g. Amdam *et al.,* 2007; Boorse & Denver, 2004; Chastel, Lacroix & Kersten, 2003; Giger *et al*., 2006; Gluckman & Hanson, 2006; Klaren *et al.,* 2007; Leimar, Hammerstein & Van Dooren, 2006; West-Eberhard, 2003). Species change in response to environmental parameters and on this point there appears to be wide-spread agreement (e.g. Müller & Newman, 2005). Therefore, any proposed biological mechanism for speciation must account for this interaction between gene function, life history traits, morphology and environmental conditions; it must also ascribe a role for the individuals that comprise a population or species in its model (Gould, 1980, 2002; Mayr, 1991).

Heterochrony has been successfully argued as the most probable process capable of producing the patterns documented in the fossil record of multi-cellular organisms as well as fitting the above criteria. 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 coordinated shape and size differences in descendant populations. In vertebrates, heterochrony has been implicated in a number of evolutionary novelties indicative of macroevolutionary change (i.e. the distinctive characteristics that warrant placing the taxon into a new genera or family), such as bipedal morphology in hominins (Berge, 20002), the marine adaptations of cetaceans (Thewissen, 1998) and the neotenic features of modern lungfish (Joss, 2006), among others (e.g. Sears *et al*., 2006; Shigetani *et al*., 2005; Svensson & Haas, 2005). Heterochrony is also implicated in many instances of microevolutionary change, which encompass the small character differences that distinguish species from each other or reflect adaptation within populations (e.g. Geist, 1998; McDonald & Smith, 1994; McNamara & McKinney, 2005; Gould, 2002).

The precise biological mechanism (or mechanisms) responsible for the initiation and implementation of heterochronic changes have not yet been determined, although genes and/or gene functions are still the target of virtually all research. Some evo-devo proponents insist that mutations within *cis*-regulatory elements control the morphological change associated with speciation (e.g. Prud'homme, Gompel & Carroll, 2007), a view contested by some traditional

geneticists (see review and critique by Hoekstra & Coyne, 2007). However, both sides seem to have forgotten that morphology is not all that must change during speciation. To cite but one example in a fish, the three-spined stickleback *Gasterosteus aculeatus* has shifted from its ancestral marine to various riverine habitats on many occasions (Hoekstra & Coyne, 2007, 1004; McKinnon & Rundle, 2002), generating noticeable changes in morphology that are the focus of virtually all research ─ even though physiological alterations to osmoregulation mechanisms are almost certainly more critical to successful freshwater adaptation.

In contrast, Müller (1990) suggested more than 15 years ago that the timing of activation in existing genes may be all that is modified in heterochrony. A hormonal mechanism to account for such activation effects on developmental genes has long been suspected to exist (e.g. McKinney, 1998). TH in particular has been implicated in heterochronic speciation in a number of taxa and the adaptive nature of its correlation to heterochrony in responding to environmental change, including osmoregulation, has been demonstrated (e.g. Biswas *et al*., 2006; Gomez-Mestre & Buchholz, 2006; Joss, 2006; McComb *et al*., 2005; Peter, Lock & Wendelaar Bonga, 2000; additional refs. in Crockford 2004, 2006). TH has been shown to be essential for early embryonic growth and development in virtually all vertebrates (Hulbert, 2000). Surprisingly, in spite of these harbingers, a broad evolutionary role for TH in heterochronic change has not been actively pursued until now.

THYROID HORMONES

General actions and effects of thyroid hormones

In vertebrates, THs are known to be an essential regulator for an astonishing range of critical development and maintenance functions, including:

-differentiation of embryonic and adult brain stem cells.
-early embryonic cell migration, differentiation and maturation.
-embryonic and postnatal somatic growth.
-embryonic and postnatal development of the brain and eyes.
-brain function and neurogenesis.
-hair growth.
-production of adrenal hormones necessary for stress response.
-skin and hair pigment production.
-development and function of the gonads.
-generation of energy in mitochondria.
-replication of mitochondrial DNA.

-regulation of cellular and whole-body metabolism.
-metamorphosis in amphibians.
-smoltification and osmoregulation in anadromous fish.
-adaptive colouration.
-mammalian hibernation.

Through a cascade of direct and permissive (ancillary) effects on regulatory genes and basic biochemical cell functions, which may also incorporate the effects of other hormones (Figure 1), THs influence virtually all biological systems from the point of conception onward (Biswas *et al*., 2006; Brown *et al.,* 2007; Chubb *et al*., 2005; Coppola *et al*., 2007; da Silva *et al.,* 2006; Flamant, Gauthier & Samarut, 2007; Forrest, 2004; Galton *et al*., 2007; Gilbert *et al*., 2007; Hadley, 2000; Hulbert, 2000; Jones, Thoemke & Anderson, 2005; Klaren *et al.,* 2007; Lemkine *et al*., 2005; Liu & Brent, 2005; Morvan Dubois *et al*., 2006; Poppe, Vlekeniers & Glinoert, 2007; Roberts *et al*., 2006; Trentin, 2006; Walpita *et al*., 2007; Wiens & Trudeau, 2006; Yoshimura, 2006; additional references in Crockford, 2004, 2006).

The chemical form and structure of the TH molecule is identical among vertebrates and even across phyla. Most TH is released from the thyroid gland as thyroxine *(*T_4), although some hormone is released with one less iodine molecule, as triiodothyronine (T_3). Most conversion of T_4 to T_3 occurs in tissues, including the brain and placenta, via deiodinase enzyme DII. Deiodinase enzymes (DI, DII, DIII) are themselves regulated by available TH levels and thus vital components of TH metabolism (Hulbert 2000). T_3 is considered the more physiologically relevant form (e.g. especially in metabolism and gene regulation) because it has a greater affinity for binding to receptors. However, it has also been demonstrated that T_4 by itself has critical effects, especially on early growth and development.

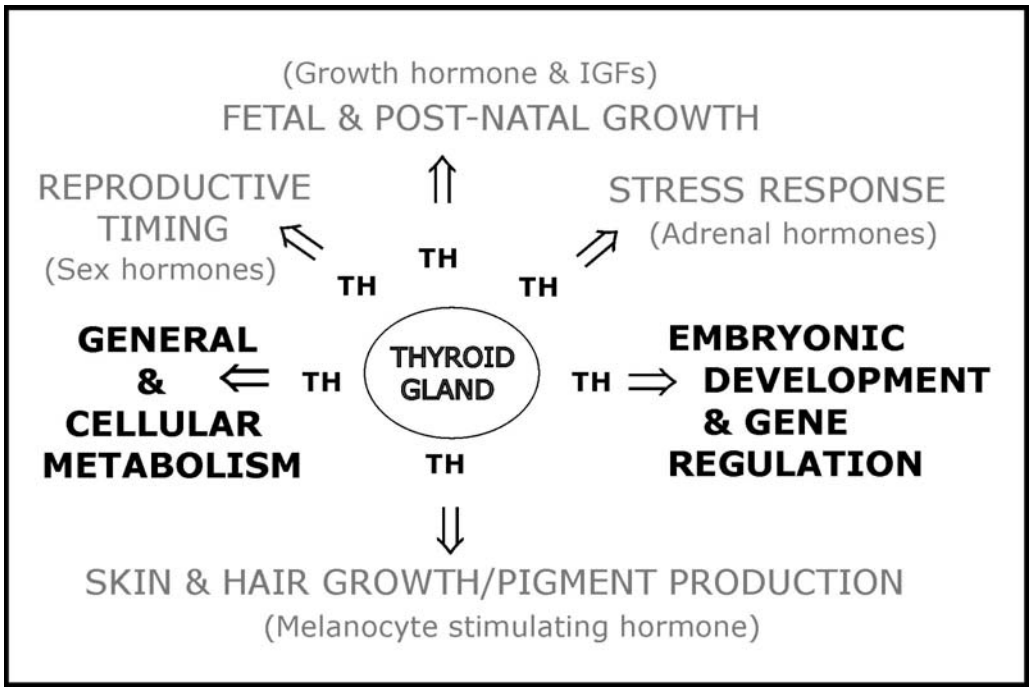

FIGURE 1. SUMMARY OF THYROID HORMONE EFFECTS ON BODY FUNCTIONS AND CHARACTERISTICS. Some effects of thyroid hormone (TH) are direct (in bold) and others are achieved via interactions with other hormones (from references in Crockford 2004, 2006).

Genomic effects of TH: gene regulation functions

As a gene regulator, T_3 binds to 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 many genes, triggering the transcription of gene products (enzymes and proteins) within cell nuclei and mitochondria. T_3 regulates the transcription of a huge number of genes, including those for nerve, bone, and epidermal growth factors (such as bone morphogenic protein, BMP4, which regulates among other things, growth of bird beaks, mammal teeth and fish mouth parts, see Abzhanov *et al*., 2004; Albertson *et al*., 2005; Kassai *et al*., 2005; Martinez & Gomes, 2005; Wu *et al*., 2004**)**, as well as critical brain and skeletal muscle function proteins, to name just a few (Clément *et al*., 2002; Roberts *et al*., 2006).

T_3 also has a critical role 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 for steroid hormone manufacture. The list of such specific effects and actions of both THs continues to grow.

In addition, T_3 is known to play a role in the epigenetic modification of genes, a process currently under consideration for having a significant impact on evolutionary change in placental mammals (e.g. Jirtle & Weidman, 2007). DNA methylation of genetic material is the result of an incompletely understood biochemical process involving several enzymes (such as histone deacetylases, HDACs, and DNA methyltransferases). Although DNA methylation often turns gene expression off or down (e.g. Rakyan *et al*., 2004; Murrell, Rakyan & Beck, 2005), it can also up-regulate expression (e.g. De Larco *et al*., 2003). The pattern of methylation for a particular gene may also be distinct in different tissues. Although methylation patterns appear to be transmissible from one generation to the next (e.g. Wolf & Hager 2006), they are also reversible (Weaver *et al*., 2004, Clément *et al*., 2002). In other words, changing methylation patterns is a way of temporarily altering gene expression. Not surprisingly, T_3 is a known regulator of several DNA modifying enzymes (Clément *et al*., 2002; Potter *et al*., 2002) and has been shown to block histone acetylation of thyroid hormone receptors (Ishizuka & Lazar 2003; Paul *et al*., 2005).

Non-genomic effects of TH: signalling functions

Some effects of T_3 do not involve the binding of the hormone to a receptor or TRE. Such non-genomic (i.e. direct) effects can be implemented rapidly (within minutes or even seconds) and underscore the essential role of T_3 to subcellular and intercellular signalling processes (da Silva *et al*., 2006; Falzacappa *et al*., 2007; Flamant, Gauthier & Samarut, 2007; Hiroi *et al*., 2006; Meaney *et al.,* 2000; Peter, Lock & Wendelaar Bonga, 2000; Wrutniak, Casa, & Cabello, 2001). Such direct actions are essential for certain developmental, cellular, and mitochondrial membrane functions, including:

-stimulation of Ca^{2+}-ATPase production in cell membranes.

-increasing oxidative phosphorylation in mitochondria.

-inducing the synthesis of Na^{+}/K^{+}-ATPase needed to activate the so-called "sodium pump" in mitochondria.

-activation of Akt phosphorylation in pancreatic islet β cells.

-inducing actin polyadenylation in somatotrophs, stimultating growth hormone synthesis and secretion.
-mediating inner ear development.
-increasing mitochondrial gene transcription of mRNA and stimulating the replication (increase in number) of mitochondria.

Thyroid hormone secretion

THs are secreted into the blood stream primarily when thyrotropin releasing hormone (TRH) from the hypothalamus induces the release of thyroid stimulating hormone (TSH or thyrotropin) by the pituitary gland, initiating TH release. In non-mammalian vertebrates, corticotropin-releasing hormone (CRH, or corticotropin) also elicits release of TSH and subsequently, TH secretion: this secondary control mechanism over TH release has been shown to give amphibians 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 (e.g. Boorse & Denver, 2004).

THs are released in a rhythmic manner in all vertebrates because the stimulus the pituitary gland receives from the brain (via TRH) is pulsatile. Thus hormone pulsatility, like circadian rhythms, originates in the suprachiasmatic nucleus (SCN) of the hypothalamus, where electrical stimulation of receptors in the retina of the eye and the central nervous system relay signals to the pineal gland and the hypothalamus. The pineal translates electrical signals into biochemical messages, producing several neurohormones, such as melatonin, serotonin, and noradrenalin. Pulses of these neurohormones prompt the hypothalamus to secrete bursts of hormone-releasing hormones, including TRH. A direct neural connection is also known to exist between the SCN of the hypothalamus and the retina in mammals. Thus, pulsatile release of pineal melatonin or direct stimulation of the hypothalamus can stimulate pulsatile secretion of TRH and subsequently, pulsatile release of THs from the thyroid. More critically, perhaps, there is now known to be a direct neural connection between the SCN and the thyroid gland itself (Kalsbeek *et al*., 2000, 2006), allowing direct and immediate stimulation of TH release by the brain that is independent of hormonal stimulation.

The precise frequency and amplitude of TH pulses are known to change both seasonally and daily according to other physiological demands (details in Crockford 2004, 2006).

Fluctuations also occur with age, reproductive stage, psychological state, and general health. As levels of TH are known to fluctuate relative to the many variables described above, static measurements (single samples) of TH and thyroid-binding protein concentrations often reported in the literature as characterizing TH function cannot be compared.

The few comparative studies that have sampled TH levels frequently enough to determine the normal daily rhythm of TH production suggest marked daily profile differences exist between closely-related species (e.g. Gancedo *et al*., 1997; Wright *et al*., 2003). Daily variations of TH concentration that result from pulsatile TH secretion have also been demonstrated in humans and other taxa (Lucke *et al*., 1977; other refs. in Crockford, 2004, 2006).

While all hormones are secreted in a pulsatile fashion and have been found to be individually variable (e.g. Windle *et al*., 1998), it is apparent from detailed study that at least some of these rhythms are not independent generated or maintained (e.g. Lincoln *et al*., 2006; Wright, 2002). Experiments in rats, birds and humans have demonstrated that THs are necessary for generating the pulsatile production of GHRH (growth hormone releasing hormone), GH (grow hormone), ACTH (adrenocorticotropic hormone or corticotropin), catecholamines, and glucocorticoids (details and refs. in Crockford, 2004). As 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, I suggest that TH rhythms may be the pacemaker that drives or regulates pulsatility in the others. While this remains to be demonstrated, there is compelling circumstantial evidence that such a central control mechanism not only exists but has substantial biological and evolutionary significance.

It has 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). This mechanism allows fully coordinated (and often rapid) modulation in such physiological traits as timing of reproductive function, osmoregulation, and increased metabolism in response to changes in light, temperature and salinity (e.g. Brown *et al.,* 2007; Chastel, Lacroix & Kersten, 2003; Coppola *et al*., 2007; de la Iglesia & Schwartz, 2006; Klaren *et al.,* 2007; Ojeda *et al*., 2006; McComb *et al*., 2005; Peter, Lock & Wendelaar Bonga, 2000; Sish & Zehr, 2006; Yoshimura, 2006). As a consequence, evidence that TH-release is distinctly pulsatile in nature and that TH rhythms vary according to both extrinsic and extrinsic factors, suggests that shifts in timing and intensity of TH pulses (i.e. changes to TH rhythms) are

the biological mechanism through which coordinated short-term adaptation is achieved in vertebrate individuals. The newly-documented dose-dependent role for THs in ontogenic growth and development at the molecular level (e.g. Gilbert *et al*., 2007; Morvan Dubois *et al*.,2006; Roberts *et al*., 2006) suggests further that TH rhythms may also be the biological mechanism responsible for allowing populations of individuals (i.e. species) to change permanently over evolutionary time.

I contend that TH rhythms 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 cellular processes), but because THs are the only known factor demonstrated so far to link (through hormonal interactions and interdependence) the morphological, reproductive, physiological and behavioural characteristics known to change in coordinated fashion during speciation. Other hormones do some of these jobs ─ only THs do them all.

Individual variation in TH metabolism

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 in insects. If so, and since all hormone rhythms tested so far are individually variable, I suggest that very slight individual variations in TH rhythms 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). In other words, individual trait differences that are noticeably evident in all populations (e.g. dominance behaviour, territoriality, stress tolerance, size at maturity, the coordinated timing of ovulation and moult, coat colors) may be regulated by a single TH rhythm variant that affects multiple genes and cellular processes simultaneously. Such a process would account for the significant individual differences in gene expression that have recently been documented in humans (e.g. Storey *et al*., 2007).

Discrete within-species alternatives of physical form, including morphotypes (as occur in certain fish, amphibian and reptile species) and ecotypes (as are more common in mammals) may also be TH-dependent. These differences often involve morph- or ecoytype-specific growth

pattern differences that are consistent within a particular population segment and are often heterochronic in nature (age- or size-specific), suggesting an underlying TH rhythm control mechanism (e.g. Leimar, Hammerstein & Van Dooren, 2006; Pitman & Ensor, 2003).

While phenotypic variation is generally assumed to exist because of slight mutational changes that occur randomly and continuously in genes (e.g. Hoekstra & Coyne, 2007; Nei, 2007), such genetic variation must be exposed, through its whole-body effects on the biochemistry, physiology, and development of the individual before it can be subjected to natural selection (Hall, 2003; West-Eberhard, 2005). A multi-tiered (hierarchical) aspect of gene expression regulated by THs as proposed here 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 appear unrelated but are biochemically, physiologically and/or developmentally linked. THs influence many characteristics in just such a complex fashion, via targeted actions on regulatory genes, cells and tissues that affect growth and development, and ancillary effects generated by interactions with other hormones. A short description of how TH regulates these phenotypic effects is provided below (details and additional refs. in Crockford, 2004, 2006).

Piebaldness and TH

Since patterning of colour is determined during early foetal development, piebaldness appears to be an inevitable consequence of heterochronic processes. Typically, colour spreads out from a dozen or more locations (vis melanoblast cells, of neural crest origin), so that areas in between that do not receive melanoblasts because of disruption of cell migration or subsequent cell proliferation remain white: piebald colouration can vary from only a spot or two of white on a solid background to an all-over white that is essentially one big spot. T_4 has been identified as essential to the orderly movement of cells out of the neural crest during early development, which is supplied by the maternal system. Consequently, although a direct correlation between disruption of maternal TH rhythms and piebaldness has not yet been demonstrated, the circumstantial evidence is very strong. The consistent appearance of piebald coat colour patterns in domesticates suggests that piebaldness may be an indirect consequence of heterochronic change.

Behaviour and TH

THs mediate behavioural responses to stress and stimuli via adrenal gland function because the production of adrenal catecholamines (i.e. epinephrine, norepinephrine and dopamine) are dependent on receptors regulated by THs. Thus, while THs are not normally regarded as "stress hormones," behaviour relating to an animal's stress response is fundamentally under TH control and just as TH rhythms show individual and intraspecific variation, so do stress responses. The ability to respond to the varied exogenous stresses which are fundamental to survival should therefore be affected by an individual's particular TH rhythm. Individual differences in TH rhythms could therefore account in part for individual differences in behavioural 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.

Development and TH

As discussed above in relation to piebald phenotypes, THs are known to be required for normal embryonic development at all stages and are 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. 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 and tooth enamel formation. In the developing central nervous system, both T_4 and T_3 have been identified as essential for oligodendroctye differentiation, axonal myelination, dendritic and axonal growth, neurotransmitter regulation and synaptogenesis (e.g. de Silva *et al*., 2006; Forrest, 2004; Jones, Thoemke & Anderson, 2005; Martinez & Gomes, 2005; Trentin, 2006; Weins & Trudeau, 2006). Recent work suggests that very minor variations of T_4 concentration in the foetus alter the development of brain cell architecture (Gilbert *et al*., 2007; Lavado-Autric *et al*., 2003) and that even minor alterations in the brain may change critical function (e.g. Mitchell, 2007). In short, a wide range of the myriad small steps that constitute the

process of embryonic development are known to be controlled by THs, including the development of the placenta itself.

Marsupials, which are born at an early stage of development, continue to receive a maternal contribution of THs via milk. In non-placental vertebrates, all required hormones and growth factors are present in the yolk, incorporated during the process of vitellogenesis. The exact amounts of essential hormones and other nutrients incorporated are controlled by the maternal system (McComb *et al*., 2005; McNabb, 2006; Walpita *et al*., 2007), which puts non-mammalian vertebrates in a similar position as placental mammals in regards to achieving maternal control over early embryonic development. Although not yet confirmed, THs are likely distributed within the yolk in layers that differ in concentration, in a manner similar to the pattern documented for steroid hormones (Bowden *et al*., 2001; Gil *et al.,* 2007), allowing the yolk to reflect maternal TH rhythms to some degree. I contend that some such concentration gradient of TH must exist in yolk because we know from experimental evidence that timing and amount of hormone delivered to TH-dependent genes in the developing embryo (such as Sonic hedgehog, fibroblast growth factor and bone morphogenic protein genes) are as critical to birds, in a threshold-dependant manner, as other vertebrates (e.g. Abzhanov *et al.,* 2004; Wu *et al.,* 2004).

This early direct role for THs is not the only aspect of its effect on development: THs also interact with the production and actions of other hormones, such as growth and sex hormones. For example, T_3 has been shown to be an essential regulator of linear growth, in conjunction with growth hormone (GH), insulin-like growth factor 1 (IGF-1), and oestrogen. GH is dependent on TH for its release from the pituitary gland and timing of reproduction is strongly correlated with TH levels (Brown *et al.,* 2007; Chastel *et al*., 2003; da Silva *et al.,* 2006). Due to the critical role played by maternal THs in early embryonic development in a time-and dose-dependent manner, it is clear that the precise endocrine physiology possessed by a mother (or passed along into egg yolk) must strongly regulate the development of her offspring from conception onward.

Nutrition and TH

The most evolutionarily critical way that nutrition can impact TH physiology is via the direct consumption of THs from food. THs are present in algae and many plants as well as in the flesh, blood, organ tissue, egg yolks and thyroid glands of all vertebrates. Unique among hormones, THs can be easily absorbed directly through the digestive tract and thus consumption

of TH-rich foods must add considerably to an animal's daily TH load, especially for carnivores and those that subsist on kelp and other algae.

In all taxonomic groups, abrupt changes in diet commonly accompany habitat shifts and increases in the relative amounts of TH-laden food in particular may contribute to the generation of developmental novelties characteristics of higher level taxa, such as new genera, families or orders, which I discuss later in relation to hominin bipedalism. Most importantly, the wide availability of consumable THs in plants and animals (including unicellular forms, such as plankton) is yet another factor that strongly implicates THs as a significant biological mechanism driving evolutionary change in multicellular organisms (e.g. Heyland & Moroz, 2005; Heyland *et al*., 2006).

Genetic control of TH rhythms

Genetic control over pulsatile TH secretion seems to come from eight or more so-called "clock genes," that reside in circadian oscillator cells of the mammalian SCN. The SCN is developed and functional by late foetal stage or the early postnatal period and the interaction of these clock 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 (e.g. Kohsaka & Bass, 2006; Kalsbeek *et al*., 2006). Since as-yet unknown "clock-modulating" genes are thought to exist in other tissues as well (e.g. Gross, 2007), the mechanism is far from being well understood. However, although circadian rhythms do modulate hormonal rhythms, the two are not identical: TH rhythms are known to shift even when light/dark cycles are constant (Wright, 2002; Yoshimura, 2006).

How do variations in TH rhythms arise? I suggest that genes controlling TH rhythm phenotypes in oscillating SCN clock neurons may accumulate mutations at a fairly rapid rate, quickly replacing variation lost during a founder event or population bottleneck: that is, the individual genes that produce TH rhythm 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 (e.g. Nei, 2007). Such mini-mutations would ensure that individual differences in TH rhythms are constantly replenished. Although this is pure speculation, it is a logical possibility that could be tested, although I discuss another option later in relation to hominin bipedalism.

A 21ST CENTURY PARADIGM – THYROID RHYTHM THEORY

The theory

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 TH rhythms (TH rhythm phenotypes) must generate the coordinated individual variation in morphology, reproduction and behaviour within populations that are 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 one particular TH rhythm phenotype), drastically reducing the total variation of TH rhythm phenotypes in the founder population. For reasons that aren't yet fully understood but which perhaps relate to the physiological stresses of the speciation process itself, this lack of TH rhythm variability generates descendants that are behaviourally, reproductively and morphologically different from the ancestral population after relatively few generations. The well-coordinated suite of differences between them are recognizable as classic heterochronic changes of one kind or another.

A new perspective on domestication

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. All domesticates are known to share certain morphological, physiological and behavioural differences from their presumed ancestors that indicate TH played a pivotal role in the heterochronic changes that occurred (details in Crockford, 2004, 2006; Dobney & Larson, 2006). Understanding how THs exert coordinated control over essential biological functions and other hormone-producing organs provides the foundation for a model that explains protodomestication as a natural speciation process rather than deliberate human innovation. This model can therefore be used to explain how all vertebrate species and higher level taxa could transform over time in response to changing environmental conditions.

The most compelling objection to domestication as an intentional human-mediated processes 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 because those traits did not exist as such in wild populations. Paedomorphic changes had to occur before human selection could be used to shape future generations. Therefore, it is imperative to examine domestication in biological terms, as an evolutionary process compared and contrasted with speciation. A few intriguing experiments have provided significant insights on this process.

Experimental selection and domestication effects

The most 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 in Siberia (Belyaev 1979; Trut *et al*., 2004; additional references in Crockford, 2004, 2006). The experiments began with a large population of farmed silver foxes, a naturally-occurring black colour morph of the red fox, *Vulpes vulpes*. These foxes retained the timid nature, coordinated annual breeding cycle (females "seasonally monoestrous") and single annual moult characteristic of the wild form.

The researchers assessed over one thousand of silver foxes and selected, as an experimental population, individuals that demonstrated somewhat less "fearful" behaviour towards people than their cohorts, a response I prefer to call "stress tolerant." The selected population comprised less than 10% of the total (130 individuals). When approached, these selected 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). Females of Belyaev's selected population of stress tolerant animals turned out to be the earliest breeders of the original total population, suggesting that an existing polymorphism for timing of oestrus correlated with stress response behaviour.

After several generations of breeding and selecting for stress tolerant behaviour, the oestrous cycle and timing of the annual moult of many females had receded in the season by several months. As the experiment continued, oestrous and moult receded further still until several females were experiencing two oestrous cycles annually, one in spring and another in fall.

After twenty generations, some females were able to produce two litters per year. This diestrous pattern of reproduction, never expressed in the original population, was found to be inherited not as a recessive characteristic, as expected, but as a dominant trait.

The totally surprising result was that after a number of generations, novel traits suddenly appeared. A classic white piebald pattern appeared in generations 8-10. After about 20 generations, some animals had a curled tail and/or drooping (flop) ears, which in wild foxes are traits of juvenile animals that disappear at maturity. All of these traits, once they had appeared in the selected population, also 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 serotonin; the pineal glands were also smaller. Females had higher levels of progesterone and oestradiol in early pregnancy accompanied by higher fertility than the original group. Subsequent osteometric analysis of selected foxes revealed that changes had also occurred in cranial conformation: skulls became shorter and broader, and the degree of sexual dimorphism was reduced.

The animals with these novel morphological and physiological traits were also described as having remarkably "dog-like" behaviour: they barked and were quite unafraid of people. This behavioural difference was evident without acclimation by the sixth generation of selection. Subsequent research indicated that none of the novel characteristics were caused by selection for particular structural genes or by spontaneous mutations.

Others researchers conducting similar experiments to this, although using different animals, got similar results. For example, Dmitriev *et al*., (2001) used an all-purpose breed of domestic chicken. They selectively bred two lines of naturally-occurring physiological phenotypes, one with particularly high (“plus”) and another with particularly low (“minus”) levels of corticosterone: two discrete selected populations (8-9 animals each) and a control. After five generations of selection for corticosterone levels only, TH and progesterone concentration levels in the “plus” line were significantly higher than the “minus” line and after only six generations, a novel phenotype (distinctive blue feathers) arose in the “plus” line.

These experiments demonstrate that selection for any one of a number of phenotypic traits in a population (including hormonal and behavioural variants) can lead to novel phenotypes that reflect a well-coordinated suite of heterochronic changes and their consequences in descendants. It is my contention that the above experiments inadvertently selected for discrete TH phenotypes,

and that known effects of THs on various biological systems in a time- and dose-dependent manner accounts for changes in behaviour, morphology, coat colour, and reproductive traits, as well as the generation of novel characteristics, in descendant populations.

Domestication as a speciation process

The model depicting domestication as a biological process (Figure 2) defines both incipient species and incipient domesticates as equivalent population subsets of wild source populations that colonize new habitats. For incipient domesticates, these environments are anthropogenic, a set of localized environmental conditions created by the effects of permanent or semi-permanent human settlement and dominated by the continuous presence or proximity of people. 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 or 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 TH rhythm phenotype) are those most likely to invade an attractive new territory.

The division of the source population during colonization of a new habitat is thus distinctly non-random. Non-random subdivision of the source population according to existing variation in TH rhythm phenotypes (and the genes that control them) makes this non-mathematical model distinct from speciation defined by the population genetic models utilized by the Modern Synthesis. 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 TH rhythms in the colonizing group. Descendants of founders are recognizable as different and reproductively isolated from their ancestors within relatively few generations because low variability of TH rhythm phenotypes within small populations has morphological, behavioural and reproductive repercussions. After the initial rapid speciation event occurs, gradual adaptation to the new habitat proceeds more slowly. While the model does not preclude the deliberate actions of people in the initial stages of protodomestication, it does not require such interference.

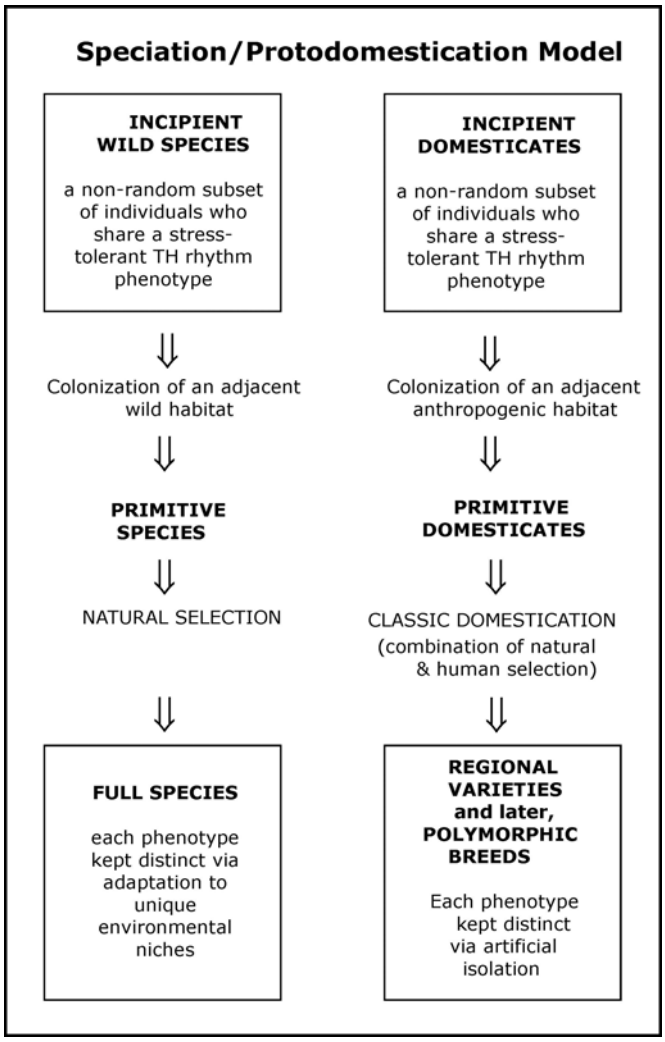

FIGURE 2. HETEROCHRONIC SPECIATION MODEL: DOMESTION AS A NATURAL SPECIATION PROCESS. The process of domestication, at least initially, is not materially different from the process that produces natural wild species. Only after the primitive domesticate has been generated via natural processes can human selection begin to shape future generations in unique ways.

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 and the selection mechanism is the same. Thus, descendants of both processes are equivalent entities that must qualify as equally real species that deserve distinct species names.

Lack of intermediate forms in all domestic taxa (based on dated skeletal remains of wild and domestic forms) indicate that protodomestication must have been exceedingly fast, almost certainly less than 200 years (Crockford, 2004, 2006; Dobney & Larson, 2006; O'Regan &

Kitchener, 2005). Not surprisingly, in Altantic salmon, *Salmo salar,* it has taken less than 25 years to transform the wild form into a distinctive domesticate, via unintentional selective mortality in response to conditions of commercial confinement: the farmed Atlantic salmon is now so different in all respects from its ancestor that some researchers suggest it be taxonomically distinguished as a new species (Gross, 1998). 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 Cichlidae in some African freshwater lakes are now estimated to have developed in as little as two hundred years (Owen, Crossley & Johnson, 1990). The “species complex” comprised of distinct marine and freshwater forms of three-spine sticklebacks may eventually prove to show similarly rapid rates (McKinnon & Rundle, 2002). Thus protodomestication and known cases of especially rapid speciation appear to operate within similar time scales.

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. In protodomestication, a shift in the timing of reproduction as well as changes in behavior of newly-transformed domesticates contribute to the cohesion of the domesticated group, establishing an effective (if not absolute) reproductive isolating mechanism. For example, reduced dominance behavior may be critical to discouraging newly-transformed domestic males from mating with ancestral females, since wild females of ancestral taxa (wolves and others) are generally unwilling to accept domestic males as mates. However, ancestral (wild) males may still have approached newly-transformed domestic females to breed, so that some introgression of wild genes (through the male lineage) probably occurred occasionally (this same unidirectional pattern of hybridization occurs in other closely-related taxa, where the new (derived) species is almost always the female partner).

Protodomestication appears to be essentially irreversible after the physical changes have occurred even if occasional introgression occurs. Eventually, the ancestral species of domestic animals (and many plants) either become extinct (e.g. dog, cattle, horse, and camel ancestors) or extremely reduced in numbers and geographic range (e.g. sheep, goat, cat, pig, Atlantic salmon ancestors), so that opportunities for introgression become increasingly rare. Once protodomestication 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" began to uniquely shape the history of newly-transformed domesticates, as I discuss elsewhere (Crockford, 2004, 2006).

Example one: speciation via colonization of a new habitat

The transformation of brown bears into polar bears is an excellent example of how the TH rhythm model devised to explain protodomestication can be applied to the generation of new wild species. Although the polar bear **(***Ursus maritimus***)** had previously been proposed as descending from ancient brown bear stock based on morphological criteria, results of genetic studies involving several mitochondrial genes (Talbot & Shields, 1996) not only confirm this conclusion but consistently place one particular coastal population of SE Alaskan brown bear (*Ursus arctos*) as a sister species to the polar bear. While no one has yet argued that polar bears are not a true, distinct species based on this evidence, their mtDNA is virtually indistinguishable from SE Alaskan brown bears. In addition, we have recently been provided evidence that hybridization in the wild does indeed occur between polar bear females and brown bear males in regions where their natural distributions overlap: a hybrid bear shot in the central Canadian arctic in April 2006 was widely reported in newspapers around the world. Talbot & Shields (1996) concluded that the morphological features that differentiate polar bears from brown bears must have evolved rapidly, probably in response to selective pressures of adapting to a new environment, and that polar bears became morphologically distinct before discrete mtDNA differences became established.

Indeed, the physical, behavioural and life history features that distinguish brown from polar bears belie their molecular similarities. Polar bears are larger than most brown bears and have a longer, narrower head. Coat colour differences are dramatic. Behaviour is also distinctive: polar bears (the derived species) are less aggressive than brown bears during interactions between them and generally less territorial, an advantage for a pelagic species that lives on drifting pack ice (Aars, Lunn & Derocher, 2005; Mauritzen, Derocher & Wiig, 2001). Polar bears are obligate carnivores while brown bears are technically omnivores (seasonally or occasionally carnivorous); their breeding seasons are distinct, although with a slight overlap.

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 shorefast and pack ice environment surrounding these refugial "islands." Such an environment would have been rich in ice-breeding ringed seals (*Pusa hispida*) – a food rarely available to animals in the source population. I propose that a few stress-tolerant brown bears (those with particular TH rhythms), colonizing shorefast and pack ice where seals high in exogenous THs were all that was available, were exposed to the essential conditions for rapid speciation to occur.

Profound changes in morphology and physiology, including the generation of a distinctive white coat, would have been inevitable within only a few generations under these conditions. Polar bears are not albino, they are the ultimate in piebaldness, essentially "one big spot." Although it may have taken many generations for the extreme piebaldness characteristic of modern polar bears to dominate the population, a less extreme black or brown and white coat must have been present in newly transformed animals. If being completely white gave polar bears any survival or reproductive advantage, partially piebald animals may have been selected against until only all-white ones remained, similar to the way in which Samoyed dogs transformed, via deliberate human selection, from a black and white animal in the late 1800's to a completely white dog less than 100 years later.

Piebaldness could only have become subject to selection because heterochronic speciation changes made it an available phenotype. Piebaldness is normally far too rare in most wild populations for selection alone (especially in a top predator like the polar bear that has no 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 substantially. Similarly, 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. On the contrary, piebaldness may simply be an inevitable consequence of small founder populations of physiologically-similar individuals colonizing radically new habitats.

Example two: speciation involving a profound diet change

Colonization of radically different environments may often have necessitated quite dramatic dietary shifts that involved adding exogenous THs sources, which may have been instrumental in generating the novelties characteristic of macroevolutionary change. Such appears to be the case in hominin evolution, where a case can be made for TH rhythm involvement in the sudden appearance of bipedal morphology. I discuss other aspects of this topic as it relates to hominin evolution elsewhere (Crockford, 2003, 2006).

Bipedalism is the earliest hominin trait noted in the African 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 hominins unique. What initiated such particular morphological changes in the first place? Various suggestions have been advanced, which assume that bipedal morphology arose because it conferred a survival or reproductive advantage, such as heat dispersal, energy efficiency, carrying infants, or using tools.

I suggest that this new morphotype was generated via heterochronic processes as a consequence of speciation, leaving some newly transformed hominins with bipedal morphology (probably with some variation in its manifestation) and some without. The skeletal traits associated with bipedal locomotion have indeed been demonstrated to be heterochronic in nature (e.g. Berge, 2002; Carroll, 2003), which suggests strongly that a significant and rapid heterochronic speciation event precipitated the changes associated with bipedalism (a speciation event almost certainly associated with colonization of a very distinctly different habitat).

I contend that the first Australopithecines (presuming these are indeed the first bipedal hominins, 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. This conclusion is supported both by isotope analysis of fossil Australopithecines confirming a diet at least as carnivorous as hyenas of that time (Sponheimer and Lee-Thorp, 1999) and by palaeontological evidence that fruit bearing trees in East Africa declined dramatically during this period (Kingdon, 2003).

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, brains, and livers (which contain especially high concentrations of THs) are generally consumed as well (Hulbert 2000). Egg yolks of all taxa also contain THs. Exogenous TH from animal prey are indistinguishable from self-produced hormone in vertebrates and THs are the only hormones readily absorbed unaltered through the digestive tract.

Consumption of large quantities of TH-laden foods (rather than occasional small amounts), day after day and month after month, such as proposed for these hominin founders, would have had a major impact on reproductive females in particular. I suggest that only those individual hominin ancestors who were relatively tolerant of high stress situations would have chosen to colonize a radically new environment in the first place, as argued for protodomestication. Even stress-tolerant colonizing females, 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 hormone "TH rhythm resilience" to distinguish it from the characteristic previously discussed, that of "stress tolerance." There is abundant evidence that exogenous T_4 crosses the placenta (in both experimental animals and modern humans) and that significant changes in normal TH levels during pregnancy have profoundly disruptive affects on the developing foetus (e.g. Poppe, Vlekeniers & Glinoert, 2007; Vaidya *et al*., 2007; additional refs. in Crockford, 2004, 2006). There is no reason to expect that incipient Australopith females would have responded differently to levels of exogenous THs that exceeded their normal intake.

I propose (based on the known requirement in all vertebrates of appropriate levels of THs at appropriate times in maintaining normal embryonic development and foetal growth) that a major and sudden shift in diet such as I have suggested for founding groups of incipient Australopiths 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. Live offspring afflicted with profound anomalies probably died at birth. However, survival rates of infants with relatively minor anomalies may have been quite high and among these could have been a suite of slight but coordinated changes 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 hormone rhythm

genes to the next generation. While this may sound like Goldschmidt's "hopeful monster" scenario, no macromutations or even macromutational effects are proposed (see Müller & Newman, 2005, 493).

If bipedal offspring had a TH rhythm phenotype similar to their mother, they would 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 TH are probably not responsible for producing the bipedal morphology specifically: rather, TH disruption likely provided an enduring selective factor which reduced the breeding population of colonizers, in each succeeding generation, to only those females with a TH rhythm resilient enough to produce viable offspring under prevailing conditions of high exogenous TH. Subsequent fine-tuning adaptation would occur through a combination of genetic and hormonal accommodation (e.g. Gomez-Mestre & Buchholz, 2006).

However, I contend there is also a possibility that disruptively high levels of exogenous TH could have permanently altered the phenotype of the newborn offspring and altered its neonatal growth program through impacts on the architecture of the oscillating cells in the SCN whose interactions control the TH rhythm. If timing and absolute amounts of TH are critical to the development of embryonic brain cellular architecture, as appears to be the case (Lavado-Autric *et al.,* 2003), disruptive amounts of exogenous THs could have impacted maternal TH rhythms to such an extent that normal embryonic migration, proliferation and maturation of SCN oscillating cells were compromised, generating slight differences in the physical relationship of these neurons to each other (e.g. Mitchell, 2007). Such a disruption could alter the combined output of SCN oscillating cells enough to produce TH rhythm differences in offspring ─ novel TH rhythms capable of permanently affecting the growth, development and behaviour of the individual and its descendants. It is therefore possible that disruptive levels of exogenous TH impacted founding populations of incipient bipedal Australopiths by providing strong selection for resilient maternal TH rhythms and through developmental effects on foetal brain architecture,

producing a macroevolutionary shift in body form accompanied by physiological and behavioural changes.

Only in vertebrates?

Is this concept applicable only to vertebrates? Taken in its broadest sense, the answer is no. A hormonal mechanism controlling development, similar to that proposed for vertebrates, also exists for invertebrates and plants. The controlling substance in insects is juvenile hormone, a molecule very similar in structure and developmental functions to both retinoic acid and TH (Flatt *et al*., 2006). There is clear evidence that such regulating hormones in insects have rhythmic secretion patterns and that heterochrony is a prevalent pattern in their evolutionary history (Nijhout, 1999). Similarly, plants also possess hormones whose interactions affect growth and development (Farnsworth, 2004) and for which clear rhythmic cycles are evident (Hall & Watters, 2005). Heterochrony has been shown to operate as a significant evolutionary process in many plant lineages.

In some marine invertebrates, including corals, sea urchins and sea stars, exogenous TH from ingested algae initiates metamorphic processes. A number of species of kelp and other algae (including unicellular phytoplankton) incorporate and store iodine (actually, inorganic iodide) as T_3, T_4 , or their precursor molecules (e.g. Heyland & Moroz, 2005), providing ready-made THs to all animals that consume them – including zooplankton, herbivorous fish, amphibians, invertebrates and some mammals. Recently, it has been shown that a sea urchin (*Lytechinus variegatus*), a sea hare (*Aplysia californica*) and at least two sand dollar species not only utilize exogenous THs but synthesize them as well (Heyland *et al*., 2006).

Some bacteria can take up, bind and degrade THs biochemically, but do not appear to utilize them as is (DiStefano, De Luze & Nguyen, 1993; Hulbert, 2000). Iodine on its own, however, is toxic to bacteria ─ hence its usefulness as an antiseptic. Bacteria thus differ significantly from unicellular organisms, such as plankton, that can ingest inorganic iodide and store it as ready-made THs: bacteria are actually more like mitochondria (the energy producing organelles found in plant and animal cells) in the way they utilize THs, although not identical. Not surprisingly, mitochondria, appear to have descended from bacteria (Embley & Martin, 2006; Gabaldón & Huynen, 2003). Mitochondria not only actively transport THs across their membranes from cell cytoplasm as bacteria do but utilize THs for many of their critical functions,

including energy production and DNA replication (Wrutniak, Casa, & Cabello, 2001). Thus, while iodine is essential to virtually all life forms, there is a clear division between those that utilize inorganic iodide and those that ingest ready-made TH.

Therefore, similarities between mitochondria and bacteria in their ability to take up exogenous THs suggests that the transformation that allowed certain bacteria to obtain iodine indirectly, from exogenous THs rather than absorbing inorganic iodide from water, was a key innovation in the evolution of animals. Only vertebrates and a few marine invertebrates are capable of independent TH synthesis (perhaps not surprisingly, the sea urchin also has remarkable genetic similarity to vertebrates (Pennisi, 2006). Although most plant and invertebrate species probably extract iodine from water and store it as TH in one form or another, few have actually been tested for this feature. Such differences suggest that THs, and the iodine necessary for their formation, may have an exceptionally broad evolutionary role in adapting life history traits to changing environmental conditions in a manner distinct from genetic mutation of specific traits, a big-picture approach that takes the model proposed here for vertebrates to a whole new level: it becomes, quite simply, a general evolutionary paradigm.

Implications for human health

It should be readily apparent that this concept has significant implications for human health: our bodies are clearly governed as much by hormones as by genes. Attempts to refute this paradigm will automatically fill holes in our understanding of TH function in all animals, including humans. As a consequence, this will improve the ability of medical practioners to provide better health care to each and every one of us as we grow, reproduce and age (e.g. Boelaert & Franklyn, 2005; MacCallum, 2007), and for assessing the total impact of chemical contaminants known to disrupt TH function.

Too much TH (hyperthyroidism) or too little (hypothyroidism) make us decidedly ill. Millions of people worldwide are currently afflicted by the symptoms of thyroid dysfunction, more than diabetes and cancer combined (Wartofsky & Dickey, 2005). Hypothyroidism is by far the most common of the two conditions and generates diverse symptoms that reflect the broad range of effects that THs have on the body, such as:

1) **Brain function:** depression; poor memory; inability to concentrate; mood swings; sleep disturbances; anxiety.

2) **Skin/hair quality:** dry skin; puffy eyes; hair loss; coarse or brittle hair.

3) **Reproductive functions:** irregular menstruation; heavy menstruation; infertility; miscarriage; birth defects; premature birth; erectile dysfunction; premature ejaculation.

4) **Circulation/respiration functions:** high cholesterol; high blood pressure; congestive heart failure; sleep-associated breathing problems.

5) **Muscle/joint function:** muscle cramps; muscle weakness; muscle and joint pain; deep voice; hoarse voice; "pins and needles" in hands/feet.

6) **Metabolic functions:** heat/cold intolerance; weight gain; constipation; general tiredness.

Hypothyroidism has also been strongly linked to hyperactivity disorders in children, to postpartum depression and some kinds of post-traumatic stress disorders (e.g. Montero-Pedrazuela *et al*. 2006; Razvi *et al*., 2007).

While I have covered this topic in detail elsewhere (Crockford, 2006), several facts are worth reiterating in relation to current research and clinical practice:

1) The individually unique, rhythmic nature of TH production is largely ignored as a factor affecting health in humans and animals: single-sample TSH values are still used almost exclusively to assess thyroid function. In addition, although documentation has been provided that rhythm variation and/or disruption in virtually all other hormones correlate with symptoms of illness and aging (e.g. Hale *et al.,* 2007; Vaidya *et al*., 2007; Wartofsky & Dickey, 2005; Watt *et al*., 2006; Young & Veldhuis, 2006), rhythm disruption in TH has not been examined. It appears that because the peaks and troughs of TH rhythms seldome fall outside broadly defined "normal" values (e.g. Stockigt, 2000), combined with the fact that THs have a relatively long half-life, TH rhythms are assumed to have no biological significance. As far as I can determine, this supposition has not been tested. There is, however, growing acceptance that individuals probably have a unique "set-point" for TH function (e.g. Peeters, van der Deure & Visser, 2006), an acknowledgement that individual differences in TH metabolism not only exist, but are biologically and medically significant.

2) Contrary to almost every known medical reference one might consult, TH secretion can be stimulated or depressed independently of changing TSH levels, due to the direct nerve connections between the SCN and the thyroid gland. The activity of these direct nerve

connections also affects the responsiveness of the thyroid gland to TSH, which means single-sample TSH measurements (the current "gold standard") do not adequately assess thyroid heath (Kalsbeek *et al*., 2000, 2006).

3) The link between stress, THs and adrenal hormones (so-called "stress hormones") is seldom made (Badyaev, 2005; Marti *et al*., 1996), which not only undervalues the critical pacemaker function of TH but ignores direct effects of diminished TH levels on tissue and cell function: stress has been shown to rapidly decrease TH production, so that while some effects of stress are clearly due to subsequent TH-mediated changes in adrenal and pituitary hormone secretion, others are direct affects of reduced TH availability to the liver, brain and reproductive organs (e.g. Meaney *et al.,* 2000). Ignoring the interrelationships between THs, adrenal hormones and stress almost certainly makes it unduly difficult to diagnose and treat stress-related illnesses effectively.

4) Because thyroid dysfunction is often found to be associated with an increase in specific antibodies, virtually all TH disorders are categorized as autoimmune disease. For example, in Graves hyperthyroidism, a temporary excess of TH (perhaps due to the system rebounding from a stress response) triggers the production of antibodies to the TSH receptor. However, while these antibodies generate continued excess TH production (i.e. hyperthyroidism), normalizing TH levels *by any means* has been shown to normalize antibody levels (Laurberg, 2006). Similarly, recent studies show that smoking not only raises TH levels in a dose-dependent manner but appears to protect against the development of thyroid peroxidase antibodies (TPO-Ab), the diagnostic marker for Hashimoto's hypothyroidism (Krassas & Wiersinga, 2006; Metsios *et al*., 2007). This suggests that low TH levels (transient hypothyroidism, perhaps due to stress) may trigger the development of TPO-Ab antibodies, which then perpetuate depressed TH levels. Bringing TH levels back up to normal in hypothyroidism may bring TPO-Ab antibody production under control in similar fashion as shown for hyperthyroidism ─ not an absurd suggestion given that THs are known to play a critical role in immune function (Klecha *et al*., 2006; Dorshkind & Horseman, 2000).

5) It has now been demonstrated that even slight variations in T_4 levels during pregnancy can affect fetal outcome, especially for brain and eye development. Some birth defects, such as Down's Syndrome, fetal alcohol syndrome, colour vision abnormalities, hearing defects and certain craniofacial malformations may prove to have a common mechanism: TH rhythm

disruption at some particular point in development (e.g. Cordero *et al*., 2004; Roberts *et al*., 2006; van Trotsenburg *et al*., 2005). The disruptive effects of chemical contaminants on TH function (both known and suspected) are of particular concern in this regard (e.g. Boas *et al.,* 2006; Veldhoen *et al.* 200).

In general, the increasing incidence of hypothyroidism seems to be keeping pace with increasing incidents of obesity, cardiovascular dysfunction, depression, diabetes and infertility (e.g. Chubb *et al*., 2005; Poppe, Vlekeniers & Glinoert, 2007; Razvi *et al*., 2007; Roos *et al*., 2007; Trivieri *et al*., 2006; Vaidya *et al*., 2007; Vargas *et al*., 2006; Watt *et al*., 2006; Young & Velduis, 2006). Since we know that THs strongly influence these body functions, the coincident pattern of rising clinical cases should be cause for concern. To reiterate, however, it is clearly not enough to look at static TH levels: the correlation, if there is one, may be in disruption of TH rhythms rather than abnormally high or low levels of TH or TSH.

Testing the hypotheses

The basic hypothesis proposed by this theory that needs to be tested is whether daily rhythmic TH secretion profiles of all vertebrate species are indeed individually variable and whether such variations between individuals (TH rhythm phenotypes) are correlated with discernable morphological, reproductive, and/or behavioural differences. Within a species, breed or particular morphotype, individual TH rhythm phenotypes averaged together should generate a distinctive pattern that is species/breed/morphotype-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 be difficult due to the dynamic nature of the endocrine system and its inherent sensitivity to stress of any kind. Such sensitivity presents a unique challenge to the determination of normal TH rhythms within and between taxa, since TH levels must be measured frequently (preferably every 5 minutes), under various controlled conditions for many individuals. However, this has been done for other hormones with success (e.g. Veldhuis, 2000; Windle *et al*., 1998), suggesting that a similar method might be suitable for testing the TH rhythm hypothesis. Unfortunately, the inability of current laboratory assays to detect THs in minute quantities (below 0.5µg/dL) places limitations on the smallest samples that can be analyzed (e.g. Abel *et al.,* 1999), which means new assay methods will be needed.

If individual variation within species-specific profiles of TH rhythms can be confirmed as a general rule, controlled selective breeding experiments will also be necessary to confirm that small interbreeding groups of physiologically-similar animals (i.e. those with similar TH rhythm profiles) produce phenotypically different descendants within 20 generations or less. If it can be demonstrated that TH rhythms are indeed variable within certain limits for different species (or breeds/morphs/ecotypes 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 patterns: the genes responsible for generating hormone pulsatility.

The essential aspect of the concept, from a scientific standpoint, is that the various hypotheses encompassed by the theory are eminently testable. However, since there are a multitude of laboratory and clinical experiments that must be undertaken before the theory becomes incorporated into an operational paradigm, I leave this step to the bright young minds of the next few decades.

DISCUSSION

The "species problem" is arguably evolutionary biology's most critical research challenge. In the context of the new hormonal paradigm presented here, understanding how new species are generated and how our own bodies function become issues of equal value. In other words, because there are profound implications for human health, TH rhythm theory makes evolution uniquely personal in a way the Modern Synthesis (as it is currently presented) simply cannot attain, no matter how eloquently its proponents argue their case (e.g. Antonovics *et al*., 2007; Avise & Ayala, 2007; Hlodan, 2007; Wilson, 2005). Without discounting the importance of antibiotic resistance, the emergence of infectious diseases or even our ability to diagnose rare genetic disorders, if biologists want more citizens (including physicians) to grasp the critical importance of evolution to society as a whole (e.g. Holden, 2006; MacCallum, 2007; Miller, Scott & Okamoto, 2006; Nehm & Reilly, 2007), they need a way of explaining to non-scientists how evolution works that resonates profoundly at a personal level. I contend that TH rhythm theory, if upheld by testing, will do just that.

CONCLUSION

Understanding the biological mechanism that produced domestic animals holds the key to unlocking the mysteries of evolutionary change, but only if we abandon the dogma that all domesticates are products of deliberate human innovation. The process of protodomestication, as I have defined and explained it here, is an appropriate model for describing the truly dynamic relationship that exists between individuals, genes and the environment that drives evolutionary change in all multi-cellular organisms. TH rhythm theory offers several testable hypotheses to address the critical issue of how distinct species-specific growth and development is achieved generation after generation, even for species with few known genetic differences between them.

I have tentatively identified individual variation in species-specific rhythms of TH production (TH rhythms) as the essential factor in vertebrates that links individual variation in a wide variety of selectable traits, including morphological, physiological, reproductive and behavioural characteristics. During colonization events, non-random population subdivision isolates small groups of stress-tolerant phenotypes as founders, which initiates the developmental changes associated with rapid heterochronic speciation in their descendants.

Disruptively high levels of exogenous THs, ingested by colonizing mothers as a consequence of habitat-dependent dietary changes, impose a selective factor for particular patterns of hormonally-programmed growth of offspring and may be capable of permanently altering their TH rhythms. This non-genetic influence over development may explain the rather sudden appearance of some evolutionary novelties that are heterochronic in nature, such as bipedalism in early hominins.

The wide-spread utilization of THs, or hormones analogous to them, in plants and invertebrate animals suggest that hormone rhythms regulate and coordinate 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, it suggests the possibility that my theory, taken in its broadest sense, might explain rapid adaptation and speciation in all multi-cellular organisms.

TH rhythm theory is based on the premise that a simple biological mechanism exists that allows species to adapt and transform over evolutionary time in response to changing environmental conditions: the premise merely expands in scope the existing system individual animals are known to use for adapting to daily and seasonal changes in their lifetime. If my

assertion is upheld—that individual variants of TH rhythm phenotypes effectively control a suite of inter-connected physiological, morphological and behavioural characteristics—then the concept describes an extremely critical reservoir of variation within populations 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 TH rhythms should produce well coordinated and rapid changes in all biological systems simultaneously, a factor that surely must be important in creating viable alternative life history traits (which are reflected in differing morphology, physiology and behaviour) in response to changing environmental conditions over evolutionary time.

I do not discount the importance or effects of genes, merely the absolute supremacy attributed to them by the Modern Synthesis in driving evolutionary change of their own accord in all types of organisms. Although THs have important non-genomic effects on development and cellular function, the impact they have on gene function is profound: since T_3 is a known transcription factor for developmentally critical genes, alterations to the timely availability of T_3 can change the expression of myriad genes without mutation. In addition, as T_3 is a known regulator of several DNA modifying enzymes, the processes of epigenetics and gene imprinting do not rule out a pivotal role for TH rhythms in evolutionary change. Indeed, gene modification may be one mechanism by which shifting TH levels implement change.

Acknowledging the enormous effects that timely availability of THs have on gene function in multi-cellular organisms allows us to describe, for the first time, a plausible biological mechanism that can operate at all levels of evolutionary change. Thus, shifting TH rhythms can drive the gradual adaptation of species or populations to local environmental conditions, a situation where natural selection of random genetic mutation may also operate (i.e. as described by the current genetic paradigm); this within population adaptation (driven by slight shifts in prevailing hormone rhythms and/or random mutation) can lead to speciation under conditions of allopatry or parapatry and comprises the degree of change signified by the term "microevolution." In addition, the rapid transformation of one species to another in response to environmental change or colonization of a new habitat can be driven by non-random founder effects (the particular TH rhythms possessed by founders); this rapid transformation can, under certain circumstances, generate evolutionary novelties characteristic of new genera and

families—these are the speciation events that occur under conditions of peripatry or sympatry, and which can generate a degree of change signified by the term "macroevolution."

The unifying hormonal paradigm described here offers a long-overdue addendum to the exclusively genocentric view mandated by the Modern Synthesis. Its novel perspective offers new hope that the "species problem" might soon be a thing of the past and provides a solution that could precipitate a 21st century transformation of evolutionary biology and medical science.

REFERENCES

Aars, J., Lunn, N. J. & Derocher A.E. (2005) *Polar Bears: Proceedings of the 14th Working Meeting of the IUCN/SSC Polar Bear Specialist Group, 20-24 June 2005, Seattle, Washington, USA*. Occasional Paper of the IUCN Species Survival Commission 32, IUCN, Gland (Switzerland) and Cambridge (UK).

Abel, E.D., Boers, M., Pazor-Moura, C., Moura, E., Kaulbach, H., Zakaria, M., Lowell, B., Radovick, S., Liberman, M.C. & Wondisford, F. (1999) Divergent roles for thyroid hormone receptor β isoforms in the endocrine axis and auditory system. *Journal of Clinical Investigations* **104**, 291-300.

Abzhanov, A., Protas, M., Grant, B.R., Grant, P.R. & Tabin, C.J. (2004) *Bmp4* and morphological variation of beaks in Darwin's finches. *Science* **305**, 1462-1465.

Albertson, R.C., Streelman, J.T., Kocher, T.D. & Yelick, P.C. (2005) Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. *Proceeding of the National Academy of Science USA* **102**, 16287-16292.

Amdam, G.V., Nilsen, K., Norberg, K., Fondrk, M.K. & Hartfelder, K. (2007) Variation in endocrine signaling underlies variation in social life history. *American Naturalist* **170**, 37-46.

Antonovics, J., Abbate, J.L., Baker, C.H., Daley, D., Hood, M.E., Jenkins, C.E., Johnson, L.J., Murray, J.J., Panjeti, V., Rudolf, V.H.W., Sloan, D. & Vondrasek J. (2007) Evolution by any other name: antibiotic resistance and avoidance of the E-word. *Public Library of Science Biology* **5**, 137-140.

Avise, J.C. & Ayala, F.J. (2007) In the light of evolution I: Adaptation and complex design. *Proceedings of the National Academy of Sciences USA* **104** (Suppl.1), 8563-8566.

Badyaev, A.V. (2005) Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society B* **272**, 877-886.

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

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

Biswas, A., Kundu, S., Roy, S., De, J., Pramanik, M. & Ray, A.K. (2006) Thyroid hormone profile during annual reproductive cycle of diploid and triploid catfish, Heteropneustes fossilis (Bloch). *General andComparative Endocrinology* **147**, 126-132.

Boas, M., Feldt-Rasmussen, U., Skakkebaek, N.E. & Main, K.M. (2006) Environmental chemicals and thyroid function. *European Journal of Endocrinology* **154**, 599-611.

Boelaert, K. & Franklyn, J.A. (2005) Thyroid hormone in health and disease. *Journal of Endocrinology* **187**, 1-15.

Boorse, G.C. & Denver, R.J. (2004) Endocrine mechanisms underlying plasticity in metamorphic timing in spadefoot toads. *Intergrative and Comparative Biology* **43**, 646-657.

Bowden, R.M., Ewert, M.A., Lipar, J.L. & Nelson, C.E. (2001) Concentration of steroid hormones in layers and biopsies of chelonian egg yolks. *General and Comparative Endocrinology* **121**, 95-103.

Breuker, C.J., Debat, V. & Klingenberg, C.P. (2006) Functional evo-devo. *Trends in Ecology and Evolution* **21**, 488-492.

Brown, J.L., Somerville, M., Riddle, H.S., Keele, M., Duer, C.K. & Freeman, E.W. (2007) Comparative endocrinology of testicular, adrenal and thyroid function in captive Asian and African elephant bulls. *General and Comparative Endocrinology* **151**, 153-162.

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

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

Chastel, O., Lacroix, A. & Kersten, M. (2003) Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus. Journal of Avian Biology* **34**, 298-306.

Chubb, S.A.P., Davis, W.A. & Davis, T.M.E. (2005) Interactions among thyroid function, insulin sensitivity, and serum lipid concentrations: the Fremantle diabetes study. *The Journal of Clinical Endocrinology & Metabolism* **90**, 5317-5320.

Clément, K., Viguerie, N., Maximillian, D., Alizadeh, A., Barbe, P., Thalamas, C., Storey, J.D., Brown, P.O., Barsh, G.S. & Langin, D. (2002) In vivo regulation of human skeletal muscle gene expression by thyroid hormone. *Genome Research* **12**, 281-291.

Coppola, A., Liu, Z-W., Andrews, Z.B., Paradix, E., Roy, M-C, Friedman, J.M., Ricquier, D., Richard, D., Horvath, T.L., Gao, X-B. & Diano, S. (2007) A central thermogenic-like mechanism in feeding regulation: an interplay between arcuate nucleus, T3 and UCP2. *Cell Metabolism* **5**, 21-33.

Cordero, D., Marcucio, R., Hu, D., Gaffield, W., Tapadia, M. & Helms, J.A. (2004) Temporal perturbations in sonic hedgehog signaling elicit the spectrum of holoprosencephaly phenotypes. *Journal of Clinical Investigation* **114**, 485-495.

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

Crockford, S.J. (2004) Animal Domestication and Vertebrate Speciation: A Paradigm for the Origin of Species. Ph.D. dissertation. University of Victoria, Canada.

Crockford, S.J. (2006) *Rhythms of Life: Thyroid Hormone and the Origin of Species.* Trafford, Victoria.

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 1883 edn. Johns Hopkins University Press, Baltimore.

de Silva, F.G., Giannocco, G., Santos, M.F. & Nunes, M.T. 2006. Thyroid hormone induction of actin polymerization in somatotrophs of hypothyroid rats: potential repercussions in growth hormone synthesis and secretion. *Endocrinology* **147**, 5777-

5785.

De Larco, J.E., Wuertz, B.R.K., Yee, D., Rickert, B.L. & Furcht, L.T. (2003) Atypical methylation of the interleukin-8 gene correlates strongly with the metastatic potential of breast carcinoma cells. *Proceedings of the National Academy of Sciences USA* **100**, 13988-13993.

Dobney, K. & Larson, G. (2006) Genetics and animal domestication: new windows on an elusive process. *Journal of Zoology* **269**, 261-271.

Dorshkind, K. & Horseman, N.D. (2000) The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. *Endocrine Reviews* **21**, 292-312.

DiStefano III, J.J., De Luze, A. & Nguyen, T.T. (1993) Binding and degradation of 3,5,3'-triiodothyronine and thyroxine by rat intestinal bacteria. *American Journal of Physiology* **264**, E966-E972.

Dmitriev, V.B., Churkina, I.V., Smirnov, A.F. & Nikolaeva, E.K. (2001) Destabilizing effect of chicken selection for the functional adrenal reserves. *Russian Journal of Genetics* **37**, 412-417.

Embley, T.M. & Martin, W. (2006) Eukaryotic evolution, changes and challenges. *Nature* **440**, 623-630.

Erwin, D.H. (2000) Macroevolution is more than repeated rounds of microevolution. *Evolution and Development* **2**, 78-84.

Falzacappa, C.V., Petrucci, E., Patriarca, V., Michienzi, S., Stigliano, A., Brunetti, E., Toscano, V. & Misiti, S. (2007) Thyroid hormone receptor TRβ1 mediates Akt activation by T_3 in pancreatic β cells. *Journal of Molecular Endocrinology* **38**, 221-233.

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

Flatt, T., Moroz, L.L., Tatar, M. & Heyland, A. (2006) Comparing thyroid and insect hormone signalling. *Integrative and Comparative Biology* **46**, 777-794.

Flamant, F., Gauthier, K. & Samarut, J. (2007) Thyroid hormones signalling is getting more complex: STORMS are coming. *Molecular Endocrinology* **21**, 321-333.

Forrest, D. (2004) The developing brain and maternal thyroid hormones: finding the

links. *Endocrinology* **145**, 4034-4036.

Futyama, D.J. (2005) The nature of natural selection. In: *Evolutionary Science and Society: Education a New Generation* (Ed. by J. Cracraft & R. Bybee), pp. 93-98. Biological Sciences Curriculum Study, Washington, DC.

Gabaldón, T. & Huynen, M.A. (2003) Reconstruction of the proto-mitochondrial metabolism. *Science* **301**, 609.

Galton, V.A., Wood, E.T., St. Germain, E.A., Withrow, C-A., Aldrich, G., St. Germain, G.M., Clark, A.S. & St. Germain, D.L. (2007) Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. *Endocrinology* **148**, 3080-3088.

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. *General and Comparative Endocrinology* **107**, 240-250.

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

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

Gepts, P. (2005) Plant and animal domestication as human-made evolution. In: *Evolutionary Science and Society: Education a New Generation* (Ed. by J. Cracraft & R. Bybee), pp. 180-186. Biological Sciences Curriculum Study, Washington, DC.

Gerstein, M.B., Bruce, C., Rozowsky, J.S., Zheng, D., Du, J., Korbel, J.O., Emanuelsson, O., Zhang, Z.D., Weissman, S. & Snyder, M. (2007) What is a gene, post-ENCODE? History and updated definition. *Genome Research* **17**, 669-681.

Giger, T., Excoffier, L., Day, P.J.R., Champigneulle, A., Hansen, M.M., Powell, R. & Largiadèr, C.R. (2006) Life history shapes gene expression in salmonids. *Current Biology* **16**, R281-282.

Gilbert, M.E., Sui, L., Walker, M.J., Anderson, W., Thomas, S., Smoller, S.N., Schon, J. P., Phani, S. & Goodman, J.H. (2007) Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. *Endocrinology* **148**, 92-102.

Gingeras, T.R. (2007) Origin of phenotypes: genese and transcripts. *Genome Research* **17**, 682-690.

Gluckman, P.D. & Hanson, M.A. (2006) Evolution, development and timing of puberty. *Trends in Endocrinology and Metabolism* **17**, 7-12.

Goldenfeld, N. & Woese, C. (2007) Biology's next revolution. *Nature* **445**, 369.

Gomez-Mestre, I. & Buchholz, D.R. (2006) Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences USA* **103**, 19021-19026.

Gould, S.J. (1980) Is a new and general theory of evolution emerging? *Paleobiology* **6**, 119-130.

Gould, S.J. (2002) *The Structure of Evolutionary Theory.* Belknap Press, Cambridge.

Grant, P.R., Grant, B.R. & Abzhanov, A. (2006) A developing paradigm for the development of bird beaks. *Biological Journal of the Linnean Society* **88**, 17-22.

Gross, L. (2007) Central and peripheral signals set the circadian liver clock. *Public Library of Science Biology* **5**, 1-2.

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,** (Supplement), 131-144.

Hadley, M.E. (2000) *Endocrinology*, 5th edn. Prentice-Hall, Inc., Englewood Cliffs.

Hale, G.E., Zhao, X., Hughes, C.L., Burger, H.G., Robertson, D.M. & Fraser, I.S. (2007) Endocrine features of menstral cycles in middle and late reproductive age and the menopausal transition classified according to the Staging of Reproductive Aging Workshop (STRAW) staging system. *Journal of Clinical Endocrinology and Metabolism* **92**, 3060-3067.

Hall, A.J.W. & McWatters, H.. (2005) *Endogenous Plant Rhythms.* Blackwell Publishing Professional, Iowa.

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.

Hansen, T.F. (2006) The evolution of genetic architecture. *Annual Review of Ecology, Evolution and Systematics* **37**, 123-157.

Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C. & Quinn, T.P. (2000) Rapid

evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**, 516.

Hendry, A. (2007) The Elvis paradox. *Nature* **446**, 147-150.

Heyland, A. & Moroz, L.L. (2005) Cross-kingdom signalling: An insight from thyroid hormone functions in marine larvae. *Journal of Experimental Biology* **208**, 4355-4361.

Heyland, A., Price, D.A., Bodnarova-Buganova, M. & Moroz, L.L. (2006) Thyroid hormone metabolism and peroxidase function in two non-chordate animals**.** *Journal of Experimental Zoology* **306B**, 551-566.

Hiroi, Y., Kim, H-H., Ying, H., Furuya, F., Huang, Z., Simoncini, T., Noma, K., Ueki, K., Nguyen, N-H., Scanlan, T.S., Moskowitz, M.A., Cheng, S-Y. & Liao, J.K. (2006) Rapid nongenomic actions of thyroid hormone. *Proceedings of the National Academy of Sciences USA* **103**, 14104-14109.

Hlodan, O. (2007) Macroevolution: evolution above the species level. *BioScience* **57**, 222-225.

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

Hoekstra, H.E. & Coyne, J.A. (2007) The locus of evolution: evo-devo and the genetics of adaptation. *Evolution* **61**, 995-1016.

Holden, C. (2006) Darwin's place on campus is secure – but not supreme. *Science* **311**, 769-771.

Hulbert, A.J. (2000) Thyroid hormones and their effects: a new perspective. *Biological Reviews* **75**, 519-631.

Ishizuka, T. & Lazar, M.A. (2003) The N-CoR/histone deacetylase 3 complex is required for repression by thyroid hormone receptor. *Molecular and Cellular Biology* **23**, 5122-5131.

Jirtle, R.L. & Weidman, J.R. (2007) Imprinted and more equal. *American Scientist* **95**, 143-149.

Jones, S.A., Thoemke, K.R. & Anderson, G.W. (2005) The role of thyroid hormone in fetal and neonatal brain development. *Current Opinion in Endocrinology and Diabetes* **12**, 10-16.

Joss, J.M.P. (2006) Lungfish evolution and development. *General and Comparative*

Endocrinology **148**, 285-289.

Kalsbeek, A., Fliers, E., Franke, A.N., Wortel, J. & Buijs, R.M. (2000) Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* **141**, 3832-3841.

Kalsbeek, A., Palm, I.F., La Fleur, S.E., Scheer, F.A.J.L., Perreau-Lenz, S., Ruiter, M., Kreier, F., Cailotto, C. & Buijs, R.M. (2006) SCN outputs and the hypothalamic balance of life. *Journal of Biological Rhythms* **21**, 458-469.

Kassai, Y., Munne, P., Hotta, Y., Penttila, E., Kavanagh, K., Ohbayashi, N. Takada, S., Thesleff, I., Jernvall, J. & Itoh, N. (2005) Regulation of mammalian tooth cusp patterning by ectodin. *Science* **309**, 2067-2070.

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

Klaren, P.H.M., Guzmán, J.M., Reutelingsperger, S.J., Mancera, J.M. & Flik, G. (2007) Low salinity acclimation and thyroid hormone metabolizing enzymes in gilthead seabream (*Sparus auratus*). *General and Comparative Endocrinology* **152**, 215-222.

Klecha, A.J., Genaro, A.M., Gorelik, G., Arcos, M.L.B., Silberman, D.M., Schuman, M., Garcia, S.I., Pirola, C. & Cremaschi, G.A. (2006) Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signalling pathway. *Journal of Endocrinology* **189**, 45-55.

Kohsaka, A. & Bass, J. (2007) A sense of time: how molecular clocks organize metabolism. *Trends in Endocrinology and Metabolism* **18**, 4-11.

Krassas, G.E. & Wiersinga, W. (2006) Smoking and autoimmune thyroid disease: the plot thickens. *European Journal of Endocrinology* **154**, 777-780.

Lacasse, J.R. & Leo, J. (2005) Serotonin and depression: a disconnect between the advertisements and the scientific literature. *Public Library of Science Medicine* **2**, 1211-1216.

Laurberg, P. (2006) Remission of Graves' disease during anti-thyroid drug therapy: time to reconsider the mechanism? *European Journal of Endocrinology* **155**, 783-786.

Lavado-Autric, R., Ausó, E., Garcia-Velasco, J.V., Carmen del Arufe, M., Escobar del Rey, F., Berbel, P. & Morreale de Escobar, G. (2003) Early maternal hypothyroxinemia

alters histogenesis and cerebral cortex cytoarchitecture of the progeny. *Journal of Clinical Investigation* **111**, 1073-1082.

Lavoie, H., Marsh, E.E. & Hall, J.E. (2006) Absence of apparent circadian rhythms of gonadotropins and free α-subunit in postmenopausal women: evidence for distinct regulation relative to other hormonal rhythms. *Journal of Biological Rhythms* **21**, 58-67.

Leimar, O., Hammerstein, P. & Van Dooren, T.J.M. (2006) A new perspective on developmental plasticity and the principles of adaptive morph determination. *American Naturalist* **167**, 367-376.

Lemkine, G.F., Faji, A., Alfama, G., Turque, A., Hassani, Z., Alegria-Prévot, O., Samarut, J., Levi, G. & Demeneix, B.A. (2005) Adult neural stem cell cycling in vivo requires thyroid hormone and its alpha receptor. *The FASEB Journal* **19**, 863-865.

Lincoln, G.A., Clarke, I.J., Hut, R.A. & Hazlerigg, D.G. (2006) Characterizing a mammalian circannual pacemaker. *Science* **314**, 1941-1944.

Liu, Y-Y. & Brent, G.A. (2005) Thyroid hormone-dependent gene expression in differentiated embryonic stem cells and embryonal carcinoma cells: identification of novel thyroid hormone target genes by deoxyribonucleic acid microarray analysis. *Endocrinology* **146**, 776-783.

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

McComb, D.M., Gelsleichter, J., Manire, C.A., Brinn, R. & Brown, C.L. (2005) Comparative thyroid hormone concentration in maternal serum and yolk of the bonnethead shark (*Sphyrna tiburo*) from two sites along the coast of Florida. *General and Comparative Endocrinology* **144**, 167-173.

McDonald, M.A. & Smith, M.H. (1994) Behavioral and morphological correlates of heterochrony in Hispaniolan palm-tanagers. *The Condor* **96**, 433-446.

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

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

McNabb, F.M.A. (2006) Avian thyroid development and adaptive plasticity. *General and Comparative Endocrinology* **147**, 93-101.

McNamara, K.J. & McKinney, M.L. (2005) Heterochrony, disparity, and macroevolution. *Paleobiology* **31** (Supplement), 17-26.

MacCallum, C. (2007) Does medicine without evolution make sense? *Public Library of Science Biology* **5**, 679-680.

Marti, O., Gavalda, A., Jolin, T. & Armario, A. (1996) Acute stress attenuates but does not abolish circadian rhythmicity of serum thyrotrophin and growth hormone in the rat. *European Journal of Endocrinology* **135**, 703-708.

Martinez, R. & Gomes, F.C.A. (2005) Proliferation of cerebellar neurons induced by astrocytes treated with thyroid hormone is mediated by a cooperation between cell contact and soluble factors and involves the epidermal growth factor-protein kinase A pathway. *Journal of Neuroscience Research* **80**, 341-349.

Mattick, J.S. (2003) Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. *BioEssays* **25**, 930-939.

Mauritzen, M., Derocher, A.E. & Wiig, Ø. (2001) Space-use strategies of female polar bears in a dynamic sea ice habitat. *Canadian Journal of Zoology* **79**, 1704-1713.

Mayr, E. (1942) *Systematics and the Origin of Species.* Columbia University Press, New York.

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

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

Mayr, E. (2001) *What Evolution Is.* Basic Books, New York.

Meaney, M.J., Diorio, J., Francis, D., Weaver, S.., Yau, J., Chapman, K. & Seckl, J.R. (2000) Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus: the effects of thyroid hormones and serotonin. *The Journal of Neuroscience* **20**, 3926-3935.

Metsios, G.S., Flouris, A.D., Jamurtas, A.Z., Carrillo, A.E., Kouretas, D., Germenis, A.E., Gourgoulianis, K., Kiropoulos, T., Tzatzarakis, M.N., Tsatsakis, A.M. & Koutedakis, Y. (2007) A brief exposure to moderate passive smoke increases

metabolism and thyroid hormone secretion. *Journal of Clinical Endocrinology and Metabolism* **92**, 208-211.

Mitchell, K.J. (2007) The genetics of brain wiring: from molecule to mind. *Public Library of Science Biology* **5**, 690-692.

Miller, J.D., Scott, E.C. & Okamoto, S. (2006) Public acceptance of evolution. *Science* **313**, 765-766.

Montero-Pedrazuela, A., Venero, C., Lavado-Autric, R., Fernández-Lamo, I., Garcia-Verdugo, J.M., Bernal, J. & Guadaño-Ferraz, A. (2006) Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. *Molecular Psychiatry* **11**, 361-371.

Morvan Dubois, G., Sebilot, A., Kuiper, G.G.J.M., Verhoelst, C.H.J., Darras, V.M., Visser, T.J. & Demeneix, B.A. (2006) Deiodinase activity is present in *Xenopus laevis* during early embryogenesis. *Endocrinology* **147**, 4941-4949.

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

Müller, G. & Newman, S.A. (2005) The innovation triad: an EvoDevo agenda. *Journal of Experimental Zoology* 304B, 487-503.

Murrell, A., Rakyan, V.K. & Beck, S. (2005) From genome to epigenome. *Human Molecular Genetics* **14**, R3-R10.

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

Nehm, R.H. & Reilly, L. (2007) Biology majors' knowledge and misconceptions of natural selection. *BioScience* **57**, 263-272.

Ojeda, S.R., Lomniczi, A., Mastronardi, C., Heger, S., Roth, C., Parent, A-S., Matagne, V. & Mungenast, A.E. (2006) The neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology* **147**, 1166-1174.

O'Regan, H.J. & Kitchener, A.C. (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review* **35**, 215-230.

Owen, R.B., Crossley, R. & Johnson, T.C. (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**, 519-553.

Pagel, M., Venditti, C. & Meade, A. (2006) Large punctuational contribution of speciation to evolutionary divergence at the molecular level. *Science* **314**, 119-121.

Paul, B.D., Buchholz, D.R., Fu, L. & Shi, Y-B. (2005) Tissue- and gene-specific recruitment of steroid receptor coactivator-3 by thyroid hormone receptor during development. *The Journal of Biological Chemistry* **280**, 27165-27172.

Peeters, R.P., van der Deure, W.M. & Visser, T.J. (2006) Genetic variation in thyroid hormone pathway genes: polymorphisms in the TSH receptor and the iodothyronine deiodinases. *European Journal of Endocrinology* **155**, 655-662.

Pennisi, E. (2003) DNA's cast of thousands. *Science* **300**, 282-285.

Pennisi, E. (2006) Sea urchin genome confirms kinship to humans and other vertebrates. *Science* **314**, 908-909.

Pennisi, E (2007) Genomicists tackle the primate tree. *Science* **316**, 218-221.

Peter, M.C.S., Lock, R.A.C. & Wendelaar Bonga, S.E. (2000) Evidence for an osmoregulatory role of thyroid hormones in the freshwater Mozambique tilapia *Oreochromis mossambicus. General and Comparative Endocrinology* **129**, 157-167.

Pitman, R.L. & Ensor, P. (2003) Three forms of killer whales (*Orcinus orca*) in Antarctic waters. *Journal of Cetacean Research and Management* **5**, 131-139.

Poelwijk, F.J., Kiviet, D.J., Weinreich, D.M. & Tans, S.J. (2007) Empirical fitness landscapes reveal accessible evolutionary paths. ***Nature*** 445, 383-386.

Poppe, K., Vlekeniers, B. & Glinoert, D. (2007) Thyroid disease and female reproduction. *Clinical Endocrinology* **66**, 309-321.

Potter, G.B., Zarach, J.M., Sisk, J.M. & Thompson, C.C. (2002) The thyroid hormone-regulated corepressor *hairless* associates with histone deacetylases in neonatal rat brain. *Molecular Endocrinology* **16**, 2547-2560.

Prud'homme, B., Gompel, N. & Carroll, S.B. (2007) Emerging principles of regulatory evolution. *Proceedings of the National Academy of Sciences USA* **104** (Suppl.1), 8605-8612.

Ptitsyn, A.A., Zvonic,S. & Gimble, J.M. (2007) Digital signal processing reveals circadian baseline oscillation in majority of mammalian genes. *Public Library of Science Computational Biology* **3**, 1-7.

Rakyan, V.K., Hildmann, T., Novik, K.L., Lewin, J., Tost, J., Cox, A.V., Andrews, T.D., Howe, K.L., Otto, T., Olek, A., Fischer, J., Gut, I.G., Berlin, K. & Beck, S. (2004) DNA methylation profiling of the human major histocompatibility complex: a pilot study for the Human Genome Project. *Public Library of Science Biology* **2**, 2170-2182.

Razvi, S., Ingoe, L., Keeka, G., Oates, C., McMillan, C. & Weaver, J.U. (2007) The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function and quality of life in subclinical hypothyroidism: randomized, crossover trial. *Journal of Clinical Endocrinology and Metabolism* **92**, 1715-1723.

Roberts, M.R., Srinivas, M., Forrest, D., Morreale de Escobar, G. & Reh, T.A. (2006) Making the gradient: thyroid hormone regulates cone opsin expression in the developing mouse retina. *Proceedings of the National Academy of Sciences USA* **103**, 6218-6223.

Roos, A., Bakker, S.J.L., Links, T.P., Gans, R.O.B. & Wolffenbuttel, B.H.R. (2007) Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *The Journal of Clinical Endocrinology & Metabolism* **92**, 491-496.

Rüber, L., Van Tassell, J.L. & Zardoya, R. (2003) Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. *Evolution* **57**, 1584-1598.

Ryan, R.G., Bloomer, P., Moloney, C.L., Grant, T.J. & Delport, W. (2007) Ecological speciation in South Atlantic finches. *Science* **315**, 1420-1423.

Salazar-Ciudad, I. (2006) Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development. *Journal of Experimental Zoology* **306B**, 107-125.

Sears, K.E., Behringer, R.R., Rasweiler IV, J.J. & Niswander, L.A. (2006) Development of bat flight: morphologic and molecular evolution of bat wing digits. *Proceedings of the National Academy of Sciences USA* **103**, 6581-6586.

Shigetani, Y., Sugahara, F. & Kuratani, S. (2005) A new evolutionary scenario for the vertebrate jaw. *BioEssays* **27**, 331-338.

Sisk, C.L. & Zehr, J.L. (2006) Pubertal hormones organize the adolescent brain and behaviour. *Frontiers in Neuroendocrinology* **26**, 163-174.

Smith, A.L., Monteiro, L., Hasegawa, O. & Friesen, V.L. (2007) Global phylogeography

of the band-rumped storm-petrel (*Oceanodroma castra,* Procellariiformes: Hydrobatidae). *Molecular Phylogenetics and Evolution* **43**, 755-773.

Smith, K.K. (2006) Craniofacial development in marsupial mammals: developmental origins of evolutionary change. *Developmental Dynamics* **235**, 1181-1193.

Sponheimer, M. & Lee-Thorpe, J.A. (1999) Isotopic evidence for the diet of an early hominid, Australopithecus africanus. *Science* **283**, 368-369.

Stockigt, J.R. (2000) Serum thyrotropin and thyroid hormone measurements and assessment of thyroid hormone transport. In: *Werner and Ingbar's The Thyroid*, 8th edn. (Ed. by L.D. Braverman & R.D. Utiger), pp. 376-392. Lippincott, Philadelphia.

Storey, J.D., Madeoy, J., Strout, J.L., Wurfel, M., Ronald, J. & Akey, J.M. (2007) Gene-expression variation within and among human populations. *American Journal of Human Genetics* **80**, 502-509.

Sullivan, J.P., Lavoué, S. & Hopkins, C.D. (2002) Discovery and phylogenetic analysis of a riverine species flock of African electric fishes (Mormyridae: Teleostei). *Evolution* **56**, 597-616.

Svensson, M.E. & Haas, A. (2005) Evolutionary innovation in the vertebrate jaw: a derived morphology in anuran tadpoles and its possible developmental origin. *BioEssays* **27**, 526-532.

Taft, R.J., Pheasant, M. & Mattick, J.S. (2007) The relationship between non-protein-coding DNA and eukaryotic complexity. *BioEssays* **29**, 288-299.

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

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

Trentin, A.G. (2006) Thyroid hormone and astrocyte morphogenesis. Journal of *Endocrinology* **189**, 189-197.

Trivieri, M.G., Oudit, G.Y., Sah, R., Kerfant, B-G., Sun, H., Gramolini, A.O., Pan, Y., Wickenden, A.D., Croteau, W., Morreale de Escobar, G., Pekhletski, R., St. Germain, D., MacLennan, D.H. & Backx, P.H. (2006) Cardiac-specific elevations in thyroid hormone enhance contractility and prevent pressure overload-induced cardiac

dysfunction. *Proceedings of the National Academy of Sciences USA* **103**, 6043-6048.

Trut, L.N., Plyusnina, I.Z. & Oskina, I.N. (2004) An experiment on fox domestication and debatable issues of evolution of the dog. *Russian Journal of Genetics* **40**, 644-655.

Vaidya, B., Anthony, S., Bilous, M., Shields, B., Drury, J., Hutchison, S. & Bilous, R. (2007) Detection of thyroid dysfunction in early pregnancy: universal screening or targeted high-risk case finding? *The Journal of Clinical Endocrinology & Metabolism* **92**, 203-207.

van Trotsenburg, A.S.P., Vulsma, T., van Rozenburg-Marres, S.L.R., van Baar, A.L., Ridder, J.C.D., Heymans, H.S.A., Tijssen, J.G.P. & de Vijlder, J.J.M. (2005) The effect of thyroxine treatment started in the neonatal period on development and growth of two-year old Down syndrome children: a randomized clinical trial. *The Journal of Clinical Endocrinology & Metabolism* **90**, 3304-3311.

Vargas, F., Moreno, J.M., Rodriguez-Gómez, I., Wangensteen, R., Osuna, A., Alvarez-Guerra, M. & García-Estañ, J. (2006) Vascular and renal function in experimental thyroid disorders. *European Journal of Endocrinology* **154**, 197-212.

Veldhoen, N., Skirrow, R.C., Osachoff, H., Wigmore, H., Clapson, D.J., Gunderson, M.P., Van Aggelen, G. & Helbing, C.C. (2006) The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development. *Aquatic Toxicology* **80**, 217-227.

Veldhuis, J.D. (2000) Nature of altered pulsatile hormone release and neuro-endocrine network signaling in human ageing: clinical studies of the somatotropic, gonadotropic, corticotropic and insulin axes. In: *Mechanisms and Biological Significance of Pulsatile Hormone Secretion* (Ed. by D.J. Chadwick & J.A. Goode), pp. 163-189. J. Wiley and Sons, Chichester.

Vrba, E.S. (2005) Mass turnover and heterochrony events in response to physical change. *Paleobiology* **31** (Supplement), 157-174.

Walpita, C.N., Van der Geyten, S., Rurangwa, E. & Darras, V.M. (2007) The effect of 3,5,3'-triiodothyronine supplementation on zebrafish (*Danio rerio*) embryonic development and expression of iodothyronine deiodinases and thyroid hormone receptors. *General and Comparative Endocrinology* **152**, 206-214.

Wartofsky, L. & Dickey, R.A. (2005) The evidence for a narrower thyrotropin reference

range is compelling. *The Journal of Clinical Endocrinology & Metabolism* **90**, 5483-5488.

Watt, T., Groenvold, M., Rasmussen, A.K., Bonnema, S.J., Hegedus, L., Bjorner, J.B. & Feldt-Rasmussen, U. (2006) Quality of life in patients with benign thyroid disorders: a review. *European Journal of Endocrinology* **154**, 501-510.

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szf, M. & Meaney, M.J. (2004) Epigenetic programming by maternal behavior. *Nature Neuroscience* **7**, 847-854.

West-Eberhard, M.J. (2003) ***Developmental Plasticity and Evolution.*** Oxford University Press, Oxford.

West-Eberhard, M.J. (2005) Developmental plasticity and the origin of species differences. *Proceedings of the National Academy of Sciences USA* **102** (Supplement 1), 6543-6549.

Whitehead, A. & Crawford, D.L. (2006) Neutral and adaptive variation in gene expression. *Proceedings of the National Academy of Sciences USA* **103**, 5425-5430.

Wiens, J.J., Engstrom, T.N. & Chippindale, P.T. (2006) Rapid diversification, incomplete isolation, and the "speciation clock" in North American salamanders (genus *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. *Evolution* **60**, 2585-2603.

Wiens, S.C. & Trudeau, V.L. (2006) Thyroid hormone and γ-aminobutyric acid (GABA) interactions in neuroendocrine systems. *Comparative Biochemistry and Physiology A* **144**, 332-344.

Wilson, D.S. (2005) Evolution for everyone: how to increase acceptance of, interest in and knowledge about evolution. *Public Library of Science Biology* **3**, 2058-2065.

Windle, R.J., Wood, S.A., Lightman, S.L. & Ingram, C.D. (1998) The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 44 rats and its relationship to differential stress responses. *Endocrinology* **139**, 4044-4052.

Wolf, J.B. & Hager, R. (2006) A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *Public Library of Science Biology* **4**, 2238-2243.

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. & Visconti, R.F. (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.

Wrutniak, C., Casa, F. & Cabello, G. (2001) Thyroid hormone action in mitochondria. *Journal of Molecular Endocrinology* **26**, 67-77.

Wu, P., Jiang, T-X., Suksaweang, S., Widelitz, R.B. & Chuong, C-M. (2004) Molecular shaping of the beak. *Science* **305**, 1465-1466.

Young, E.A. & Velduis, J.D. (2006) Disordered adrenocorticotropin secretion in women with major depression. *Journal of Clinical Endocrinology and Metabolism* **91**, 1924-1928.

Yoshimura, T. (2006) Molecular mechanism of the photoperiodic response of gonads in birds and mammals. *Comparative Biochemistry and Physiology A* **144**, 345-350.

Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K. & Ebihara, S. (2003) Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. *Nature* **426**, 178-181.