system) with qualitatively and quantitatively different and graduated positive effects. Although sometimes these may conspire to produce groups with a degree of within-population cohesion and between-population divergence that would have satisfied Ernst Mayr, there is no reason that this always or even often has to be so.

Much of the literature proceeds from the notion that species are real — if for no other reason than that systematists and ecologists seem to need them [6] — and will take any level of clustering as evidence. But no reasonable population model would have the data lack all structure. So unless there is some general agreement in advance about what degree of clustering Mayr (or we) would require, there is no principled way to decide whether papers like that of Sheppard *et al.* [1] really speak to us about 'species', 'speciation' and 'despeciation'.

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DOI: 10.1016/j.cub.2008.05.029

Chordate Metamorphosis: Ancient Control by Iodothyronines

A new study shows that iodothyronines induce metamorphosis in the cephalochordate amphioxus by binding to a receptor homologous to vertebrate thyroid hormone receptors. Iodothyronine-induced metamorphosis may be an ancestral feature of the chordates.

Robert J. Denver

A complex life cycle, where an animal begins life as a larva then undergoes a metamorphosis to the juvenile adult form, is a widespread and ancient life history strategy [1]. Larvae generally exploit different ecological niches from adults, thus avoiding competition for resources. There is considerable morphological diversity among larvae and the transformations that they undergo during metamorphosis [1], which raises the question whether the complex life cycles of extant species reflect an ancestral or a derived state.

Among chordates with complex life cycles, the best studied are the anuran amphibians (frogs and toads). Anuran larvae (tadpoles) are aquatic, and undergo morphological, biochemical and physiological transformation into the terrestrial juvenile adult. Gudernatch [2] first showed that vertebrate thyroid glands contain an active component that induces precocious metamorphosis when fed to tadpoles. Thyroid hormone is now known to orchestrate the diverse morphological and physiological changes that occur during amphibian metamorphosis [3]. Thyroid hormone has also been shown to control flatfish metamorphosis [4], and exogenous thyroid hormone can induce metamorphosis in echinoderm larvae [5–7]. Now Paris *et al.* [8] have reported in *Current Biology* that metamorphosis of the cephalochordate amphioxus can be induced by iodothyronines.

The active component in vertebrate thyroid glands is 3,5,3′5′-tetraiodothyronine (thyroxine; T_4) , a member of a class of compounds known as iodothyronines, which are derived from two tyrosine residues of a precursor protein which have iodine atoms attached to the aromatic rings [3]. Thyroxine is generally considered to be a secondary precursor that must be converted to the biologically active form of the hormone, 3,5,3′-triiodothyronine (T_3) [9]. The actions of thyroid hormone are mediated by thyroid hormone receptors, which are ligand-activated transcription factors belonging to the steroid hormone receptor superfamily [10]. All jawed vertebrates that have been studied possess two thyroid hormone receptors, TRα and TRβ, which bind to DNA as dimers, with the preferred configuration being a heterodimer with retinoid X receptor (RXR) [11]. The thyroid hormone receptors have been shown to be essential for metamorphosis of the clawed toad *Xenopus laevis* [12].

Thyroid hormone controls metamorphosis in vertebrate species, but the evolutionary origin of this developmental signaling in chordates is unknown. Most extant urochordates and cephalocordates have a complex life cycle [13]. The cephalochordate amphioxus, now considered to be among the most basal members of the phylum Chordata [14], have larvae that are asymmetric, with the mouth on the left side, and gill slits on the right side of the body (Figure 1; reviewed in [13]). At metamorphosis the pelagic larva transforms into a benthic adult. The mouth moves medially from its left lateral position, and the primary gill slits move from right to left. A secondary set of gill slits develop simultaneously on the right side of the animal. Iodothyronines are produced by the endostyle of amphioxus, a structure considered the precursor of vertebrate thyroid follicles [13,15]. Over 40 years ago, it was hypothesized that amphioxus metamorphosis is controlled by iodothyronines; however, only one attempt was hitherto made to address this hypothesis, with results complicated by an incomplete experimental design [16].

In their new work, Paris *et al.* [8] found that precocious metamorphosis in larval amphioxus is induced by treating with T_3 or T_4, although T_3 was

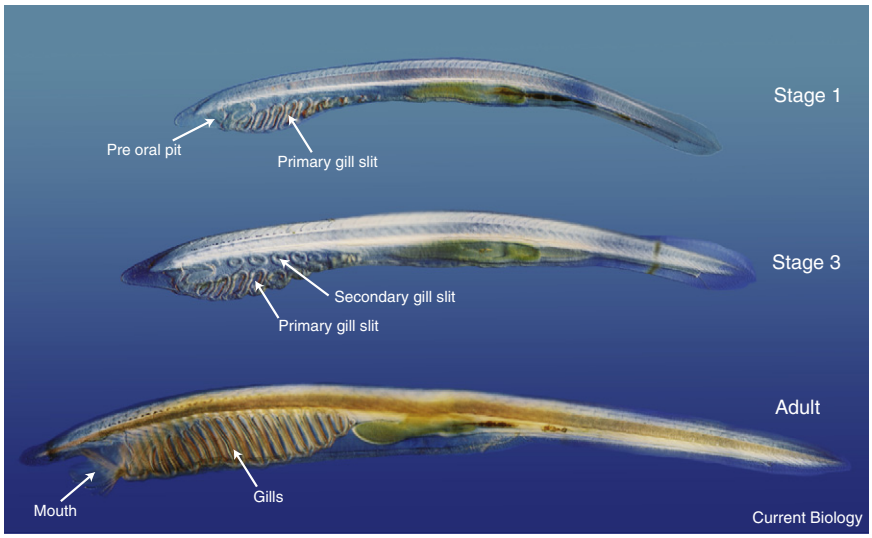

Figure 1. Metamorphosis of amphioxus.

Larval stages 1 and 3, and an adult amphioxus [13]. The pre oral pit of the early larva develops into a mouth, which moves medially from its left lateral position during metamorphosis. The primary gill slits move from right to left, and a secondary set of gill slits develops simultaneously on the right side of the animal (photos courtesy of M. Paris).

more potent than T_4. Importantly, metamorphosis could be blocked by the TR antagonist NH3. They also searched the amphioxus genome and found genes that encode proteins with sequence similarity to vertebrate enzymes involved in thyroid hormone metabolism, and a single homologue (amphiTR) of vertebrate thyroid hormone receptor genes. The authors isolated a full-length cDNA for amphiTR and found that the encoded protein can bind to thyroid hormone response elements in DNA *in vitro*, as a homodimer or a heterodimer with amphioxus RXR. Despite their earlier finding that T_3 had biological activity in amphioxus *in vivo*, however, they were unable to detect T_3-binding activity in nuclear extracts of amphioxus tissues, or with recombinant amphiTR *in vitro*. Furthermore, T_3 could not activate amphiTR in a transient transfection assay conducted in a mammalian cell line. The authors note that, in the ligand binding domain of amphiTR, two of three amino acid residues known to be important for thyroid hormone binding differ from the equivalent residues of human TRα. From this they concluded that amphiTR is not a T_3 binding protein, but the fact that T_3 can induce metamorphosis suggests that amphioxus does have a functional thyroid hormone receptor that mediates the actions of T_3 or its metabolites *in vivo*.

The findings suggested two alternative hypotheses: first, that a protein other than the amphiTR mediates the actions of T_3 on metamorphosis; or second, that T_3 is not the endogenous ligand for amphiTR, but that exogenous T_3 is converted to the endogenous ligand by amphioxus cells. Upon testing several iodothyronines Paris *et al.* [8] discovered that triiodothyroacetic acid (TRIAC) is capable of binding to and activating amphiTR. Importantly, treatment with TRIAC induced metamorphosis in amphioxus, and there is evidence that amphioxus produces TRIAC. Thus, the findings support the conclusion that amphiTR can mediate a hormonal signal, and that the endogenous ligand is not T_3, but instead TRIAC or perhaps a closely related iodothyronine.

A very early gene regulation event caused by T_3 during amphibian metamorphosis is the upregulation of TRβ [17], a phenomenon known as receptor autoinduction [18]. Autoinduction depends on thyroid hormone receptors binding to thyroid hormone response elements in the TRβ gene, and is thought to be essential for driving metamorphosis [18]. Paris *et al.* [8] found that expression of amphiTR mRNA increased during spontaneous metamorphosis, and could be induced by T_3 or TRIAC. They also showed that the 5′ flanking region of the amphiTR gene contains a putative thyroid hormone response element. Thus, receptor autoinduction likely existed in the earliest chordates and has been maintained by natural selection owing to its importance for the gene expression programs that drive metamorphosis.

These new findings show that hormone signaling mediated by thyroid hormone receptors is phylogenetically ancient, and suggest that iodothyronine-induced metamorphosis is an ancestral feature of the chordates. However, the hormone conveys no detailed instructions to particular cells, but acts to turn on or off sets of genes that underlie specific developmental processes. The number and types of genes that are regulated by iodothyronines, and variation in their temporal and spatial expression patterns likely underlie the morphological diversity seen among different taxa. Thus, within a taxonomic group, a core set of regulators comprising nuclear hormone receptors and their ligands initiate life history transitions [19], but the generation of morphological diversity may have depended on selection for the types and nature of regulation of the downstream targets of the core regulators.

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DOI: 10.1016/j.cub.2008.05.024

Circadian Rhythms: Timing the Sense of Smell

It is well established that *Drosophila* olfaction is under circadian control, yet the mechanisms underlying this temporal regulation have remained elusive. The kinase GPRK2 has now been identified as a critical link between the circadian clock and olfactory responses.

Patrick Emery and Michael Francis

Most animals use specific behavioral strategies to cope with daily changes in temperature, light intensities, and their ecological environment. For example, some species adopt a diurnal or a nocturnal behavior, while others are mostly active at dawn or dusk to avoid temperature extremes. These behavioral adaptations are largely under the control of cell-autonomous circadian clocks found in brain pacemaker neurons. These molecular oscillators are composed of a transcriptional feedback loop with a period adjusted to 24 hours by post-translational regulation of the activity and stability of its components [1]. Since most bodily functions, as well as behavior, require proper synchronization to maximize resource utilization while minimizing energy expenditure, circadian clocks are also found in many peripheral organs and tissues, where they control local physiology and metabolism [2].

Olfaction is essential for various behaviors influenced by circadian clocks (e.g., feeding and social interactions, such as mating and predator avoidance) and is itself under circadian regulation in mammals and insects [3–5]. The *Drosophila* olfactory system provides a powerful model system to explore circadian regulation of sensory processing. *Drosophila* olfactory sensory neurons (OSNs) are located in two pairs of specialized sensory organs, the antennae and the maxillary palps. Between one and four OSNs are grouped into sensilla, which are specialized hairs that dot the sensory organs, and can be classified into three types — trichoid, basiconic, and coelomic — on the basis of morphological criteria [6]. The stereotyped arrangement of the olfactory system has made it amenable to electrophysiological recordings. The electroantennogram (EAG) provides a measure of the compound response of all antennal sensilla to a given odor stimulus, and extracellular-sharp-electrode recordings from individual sensilla have provided exquisitely detailed information about the response properties (dendritic spiking activity) of single OSNs. EAG recordings demonstrated that odor responses in *Drosophila* antennae are regulated by cell-autonomous circadian clocks present within the OSNs [5,7]. The EAG amplitude changes over the course of the day, with a marked increase during the middle of the night. Interestingly, this corresponds to the time at which flies are behaviorally most reactive to odorant stimuli [8].

So, how does circadian regulation of odor sensation occur at the level of the sensory neurons? Two papers from Hardin and colleagues [9,10], in a recent issue of *Current Biology*, shed light on these issues. The olfactory response is initiated by the binding of volatile compounds to olfactory receptors (ORs). In mammals and *Caenorhabditis elegans*, ORs belong to the family of G-protein-coupled receptors (GPCRs), characterized by having 7 transmembrane domains [11,12]. Since *Drosophila* ORs have a similar membrane topology [13,14], Tanoue and colleagues [10] decided to determine whether genes known or predicted to regulate GPCR function in *Drosophila* are under circadian regulation in antennae. G-protein receptor kinases (GPRKs) were attractive candidates for these GPCR regulators. Indeed, in mammals, GPRKs phosphorylate activated GPCRs. Arrestins then bind to the phosphorylated receptors, uncouple them from their G-protein partners and, thus, terminate GPCR signaling [15]. Consistent with this idea, Tanoue *et al.* [10] found that the expression of a *Drosophila* GPRK, *gprk2*, is controlled by the circadian clock [10]. Unexpectedly, however, the levels of *gprk2* mRNA and protein oscillate in-phase with the daily oscillations in EAG amplitude, suggesting a direct correlation between GPRK2 abundance and EAG amplitude, rather than an inhibitory role for GPRK2 in OR signaling. This hypothesis was verified by measuring EAG amplitude in mutant flies with constantly high or low GPRK2 expression. Surprisingly, the daily variations in GPRK2 levels are quite modest, with night-time levels rising to concentrations that are only 1.5–2 fold greater than GPRK2 levels during the day. Increasing or decreasing GPRK2