

Snakes and ladders: the ups and downs of animal segmentation

Segmentation, the division of the body into repetitive modular subunits or metameres, is ubiquitous throughout the animal kingdom. This morphological motif appeared several times in widely divergent phyla, some without common segmented ancestors (Bateson 1894; Willmer 1990). Segmentation presents challenges to standard evolutionary narratives (Minelli and Fusco 2004), in part because segments are discrete structures, like rungs in a ladder, that are added or subtracted in an all-or-none fashion, and also because large changes in segment number can occur in evolutionary lineages with little sign of intermediate forms. Two recent papers, one on snakes (Gomez *et al.* 2008) and one on centipedes (Vedel *et al.* 2008), shed some light on these important questions.

In vertebrates, segmentation takes the form of *somitogenesis*, in which paired blocks of tissue known as somites bud off at regular time-intervals from the presomitic mesoderm (PSM) that flanks the notochord and proceed to give rise to vertebrae, ribs, muscle and dorsal dermis (Dequéant and Pourquié 2008). The numbers of segments in mammals, birds and fish are not very different, all falling well under 100, within a factor of 2 of each other. Some other groups, such as snakes, however, stand out by possessing an enormous number of vertebrae (130-500, compared to 65 in mouse, 55 in chicken, 33 in human and 31 in zebrafish; Vonk and Richardson 2008; Marx and Rabb 1972). While there has been much speculation as to how this atypical (for vertebrates) segmental phenotype may have conferred adaptive advantages to snakes and their ancestors (Willmer 1990), it seems remarkable, particularly in the context of the incrementalist scenarios favoured by the standard selectionist framework, that generation of such an extreme morphology was even attainable. The developmental dynamics disclosed in the snake and centipede studies show vividly how evolution of form can take abrupt turns.

First, Gomez *et al.* (2008) showed that their experimental animal, the corn snake, makes its somites in a fashion similar to that of fish, birds and mammals. As previously predicted by Cooke and Zeeman (1976) and later shown experimentally by Olivier Pourquié and his colleagues (reviewed in Dequéant and Pourquié 2008), the molecular-genetic mechanism that underlies this process consists of a biochemical oscillator (known as the segmentation clock) and a gradient, or wavefront. The clock is now known to comprise the periodic expression of Notch pathway signalling components and, depending on the vertebrate class, Wnt and fibroblast growth factor (FGF) pathway components as well (Dequéant and Pourquié 2008). The wavefront, with its source at the tailbud, consists, at a minimum, of FGF8 (Dequéant and Pourquié 2008). The FGF gradient serves as gate for the formation of the somites in the following fashion: the PSM, which is locally synchronous with respect to the clock, reacts to attaining a specific clock-value (i.e. a critical concentration of one of the periodically changing components) by creating a fissure, but the tissue only does this when it is located at a point of the embryo's axis where the FGF8 concentration is below a critical value. Because of the factor's graded distribution, this position is substantially anterior to the tailbud. As the tailbud grows caudally, the shallow end of the gradient regresses in the same direction, progressively allowing new blocks of the PSM to bud off from the as-yet unsegmented region when the critical clock-value next recurs in the newly disinhibited tissue.

The snake embryo exhibited cyclic expression of Lunatic fringe (*lfn*), an enzyme of the Notch signalling pathway, as well as an FGF gradient (Gomez *et al.* 2008). The wavefront in snake embryos regressed caudally by one somite length every time a somite was formed, similar to what is observed in chicken, mouse and zebrafish models of somitogenesis (Dequéant and Pourquié 2008; Holley 2007). Thus

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the key elements of the clock-and-wavefront mechanism were found to be conserved between snakes and their short-bodied vertebrate counterparts.

With its segmentation clock lacking oscillatory dynamics of Wnt and FGF pathway genes (Gomez *et al.* 2008) the snake was most similar to the zebrafish of the other species analysed, although it ultimately formed ten times as many segments. Given the conservation of the basic segmentation mechanism, several possibilities exist for the high number of somites in the snake relative to the other species.

Under the assumption (based on findings in the other species) that a somite in corn snake embryos buds off for every full turn of the clock, one possibility was that the corn snake oscillator runs faster than the others. But this did not turn out to be the case: the period of the snake embryo's clock was 100 min, compared to 120 min in mouse, 90 min in chicken and 30 min in zebrafish. Another possibility, that generation of a greater total number of PSM cells might account for greater somite number in snakes, was discounted by calculating the number of PSM cell generations, which turned out to be only slightly higher in snakes (~21 generations) than in mice (~17 generations) or chickens (~13 generations) (Gomez *et al.* 2008).

One time-dependent process that was indeed found to distinguish the snake from the other species was the *rate* of PSM cell generation, which was considerably slower than that in chickens, mice and zebrafish. But this by itself could not account for the greater number of somites. The lizard *Aspidooscelis uniparens*, for example, which forms fewer somites than the corn snake, has a comparably slow PSM cell generation rate (Gomez *et al.* 2008). The period of the lizard's segmentation clock, however, at ~4 h, is much longer than that of all the other vertebrates measured.

These comparative findings led Gomez and coworkers to formulate the hypothesis that it is the ratio of clock period ("typical" in the corn snake) to PSM growth (very slow in the snake) that leads to a high number of segments, perhaps because the wavefront regresses so slowly under these conditions that the critical clock-value is attained more frequently in a given length of tissue. In cases where the clock period and the rate of PSM growth are both typical, as in mammals, birds and fish, or both very slow, as in the lizard, a "normal" number (<100) of somites would form.

Snakes and lizards are descended from a common squamate ancestor, one with a somite number close to that of modern lizards. Based on the hypothesis of Gomez *et al.* (2008), one could speculate that this ancestor had a PSM cell cycle rate similar to modern squamates, but that the segmentation clock sped up during the evolution of the snake lineage. One could, for example, imagine a series of genetic changes affecting the characteristic rates of the regulatory networks underlying the oscillator. However, the fact that the segmentation clock is a dynamical biochemical system suggests that segmentation could also exhibit environment-dependent plasticity. Indeed, egg incubation temperature is known to affect vertebrae number in snakes (Osgood 1978), and such effects¹ might be expected to have been even more pronounced early in evolution, before canalisation of the morphological phenotype set in (Newman 2005). But we do not need to travel back in time to appreciate the interplay between plasticity of the morphological phenotype and selection in the phylogeny of metamerism. The recent field and laboratory study of the centipede, *Strigamia maritima*, by Vedel *et al.* (2008), is a case in point.

The arthropods, like chordates (including the vertebrates discussed above) and annelids, are characterised by a segmented body plan. The mechanisms employed to generate segments differ from one arthropod to another (Chipman and Akam 2008). *Drosophila*, the most famous case, employs a long germ band mode: all its segments appear more-or-less simultaneously. Centipedes are short germ band arthropods, adding their segments in a temporal sequence from a posterior growth zone. Short germ band segmentation is the more pervasive mode among the arthropods. Significantly, recent work on metamerism in spiders (Schoppmeier and Damen 2005), cockroaches (Pueyo *et al.* 2008) and centipedes (Chipman and Akam 2008) has shown that short germ band segmentation, like the sequential process of vertebrate somitogenesis, also depends on Notch signalling pathway dynamics.

Vedel and coworkers drew on earlier observations (Kettle and Arthur 2000) that within-species variation in segment number in *S. maritima* forms a geographical cline, with southern populations (in the northern hemisphere) having a greater number of segments than northern ones. This phenotypic variation is entirely

¹Fowler (1970) reviews many cases of environmental parameters such as aqueous salinity, light rhythms and oxygen concentration, as well as temperature, affecting development of meristic (repetitive) characters in vertebrates.

developmental: *Strigamia* species, unlike some of their many-legged cousins such as lithobiomorph centipedes and millipedes, do not add segments post-embryonically. By culturing eggs in the laboratory at different temperatures from clutches obtained at various sites along *S. maritima*'s geographical range, they show that higher temperatures lead to higher numbers of segments. Temperature must be acting on aspects of the segmentation mechanism other than the rate of tissue growth, since it had been previously determined that segment number and body length are not correlated in this species: individuals with a greater number of segments are more finely subdivided, rather than longer, than individuals with fewer segments (Kettle and Arthur 2000).

Although other centipede species exhibit geographic clines that parallel that of *S. maritima*, Vedel and coworkers take care to point out that plasticity cannot account for segment-number differences between different centipede *species* inhabiting different temperature zones as each species appears to have a characteristic developmental reaction norm (Arthur and Kettle 2001). The evolutionary scenario they favour is “genetic accommodation” (West-Eberhard 2003) of the phenotype acquired as a result of the temperature-dependent plasticity and subsequent canalisation (Waddington 1957), resulting in the loss of the very plasticity that allowed the new phenotype to occur.

Taken together, the findings by Gomez *et al.* (2008) on the developmental mechanism of corn snake somitogenesis and those by Vedel *et al.* (2008) on the ecological determinants of development in centipede segmentation, provide insights not only into the evolution of segmentation mechanisms, but of animal form in general. The “variational” properties of developmental mechanisms have been suggested to define potential trajectories for the evolution of morphological characters rather than merely constraining them (Salazar-Ciudad 2006). The paper by Gomez and coworkers exemplifies this by showing how a change in the ratio of two developmental parameters, the segmentation clock period and the cell generation rate of the presomitic mesoderm, leads to a greatly increased number of somites in the snake, relative to other vertebrates. Even if the variation in the ratio has a significant genetic component, the role of physical processes (i.e. synchronised oscillations of the segmentation clock and the regressing wavefront of morphogen gradients) in bringing about large-scale phenotypic changes, is clear.

If embryos are indeed partly shaped by physical processes, the forms they attain will necessarily be sensitive to their external surroundings. The paper by Vedel and coworkers confirms that the evolution of a phenotype can only be properly understood in the context of the environment in which it is expressed (Lewontin 2000). In particular, environmental parameters have the ability to modulate the molecular-physical processes that generate characters. Since the environment itself can undergo rapid change in time and space, and the characteristic response functions of such effects are often discontinuous and abrupt (Forgacs and Newman 2005), we should not be surprised that phenotypic innovation (e.g. adding or subtracting a full segment, or many at once) may not follow a gradualistic scenario (Minelli *et al.* 2009). In such cases, natural selection, rather than producing novel forms by incremental steps over long periods, would serve to stabilise novel variants, rendering them heritable within their ecological niches.

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