

Dollo's law and the re-evolution of shell coiling

Rachel Collin^{1*} and Roberto Cipriani²

¹Smithsonian Tropical Research Institute, Unit 0948, APO AA 43002, USA

²Departamento de Estudios Ambientales, Universidad Simón Bolívar, A. P. 89000, Caracas 1080, Venezuela

Gastropods have lost the quintessential snail feature, the coiled shell, numerous times in evolution. In many cases these animals have developed a limpet morphology with a cap-shaped shell and a large foot. Limpets thrive in marginal habitats such as hydrothermal vents, the high-energy rocky intertidal areas and fresh water, but they are considered to be evolutionary dead-ends, unable to re-evolve a coiled shell and therefore unable to give rise to the diversity seen among coiled snails. The re-evolution of a coiled shell, or any complex character, is considered unlikely or impossible (Dollo's law) because the loss of the character is followed by the loss of the genetic architecture and developmental mechanisms that underlie that character. Here, we quantify the level of coiling in calyptraeids, a family of mostly uncoiled limpets, and show that coiled shells have re-evolved at least once within this family. These results are the first demonstration, to our knowledge, of the re-evolution of coiling in a gastropod, and show that the developmental features underlying coiling have not been lost during 20–100 Myr of uncoiled evolutionary history. This is the first example of the re-evolution of a complex character via a change in developmental timing (heterochrony) rather than a change in location of gene expression (heterotopy).

Keywords: gastropod; calyptraeid; atavism; evolutionary reversal

1. INTRODUCTION

The palaeontological observation that morphological characters that have been lost in an evolutionary lineage are not subsequently regained has been codified as Dollo's law (Gould 1970). Although originally based on palaeontological observations of evolutionary patterns, current understanding of the genetic and developmental pathways that underlie any complex character suggests a process by which evolutionary reversals are unlikely (Marshall *et al.* 1994). The idea is that without selective forces that act to maintain their integrity when a character is expressed, the genetic and developmental pathways leading to the expression of that character will degenerate through the accumulation of mutations when the character is no longer expressed. Calculations of the probability of such mutations suggest that silenced genes may retain their function for up to 6 Myr with a reasonable probability, but that retention of function in a silenced gene for more than 10 Myr is not possible (Marshall *et al.* 1994). The few documented cases of the re-evolution of complex characters, such as wings in stick insects (Whiting *et al.* 2003) and molar teeth in *Lynx* (Kurtén 1963; Werdelin 1987), are all cases in which the structural genes are proposed to have been retained by functioning in the formation of other similar structures in a different location. The genetic architecture responsible for wing formation in stick insects is thought to have been maintained over millions of years due to its function in leg development (Whiting *et al.* 2003) and the structural genes required for *Lynx* molar development are retained by their function in the development of the other teeth.

The regular, log-spiral, coiled shell is not only the iconic image of gastropod molluscs but it is also one of the

defining characteristics of this diverse and abundant class of invertebrates. Despite several proposed advantages of the coiled shell, numerous clades of gastropods have independently lost regular shell coiling (Vermeij 1987). Groups of limpets like fissurellids (keyhole limpets), hipponicids (hoofshells), siphonarids (false limpets) and calyptraeids (slipper limpets) have all lost shell coiling as they evolved an inflated, cap-shaped shell. Other groups, like the worm snails (vermetids, vamicularids and silicularids), grow irregularly shaped twisting tubes instead of showing well-ordered coiling. It has been suggested that both types of uncoiled shells are evolutionary dead-ends because, once they have uncoiled, they are constrained to a small set of possible morphologies by an inability to re-evolve coiling (McLean 1981). The only species that may seem to have re-evolved regular shell coiling, one species of fossil worm snail, *Petalococonchus sculpturatus*, has been shown to have made a valiant attempt but ultimately failed to form a uniform log-spiral shell like their ancestors (Gould & Robinson 1994). Because there are no convincing cases of re-evolution of shell coiling (Ponder & Lindberg 1997), it has been suggested that developmental and constructional constraints prevent the re-evolution of regular coiling (Gould & Robinson 1994). Below, we demonstrate that this is not always true.

2. MATERIAL AND METHODS

(a) Study organism

The Calyptraeidae (slipper shell limpets, cup-and-saucer limpets and hat shells), a family of sedentary, filter-feeding gastropods, is primarily composed of species with uncoiled or slightly curved shells (figure 1). Out of the approximately 200 species in the family, only 10–12 species in the genera *Trochita*, *Sigapatella* and *Zegalerus* appear to be coiled. It has been assumed that

* Author for correspondence (collinr@naos.si.edu).

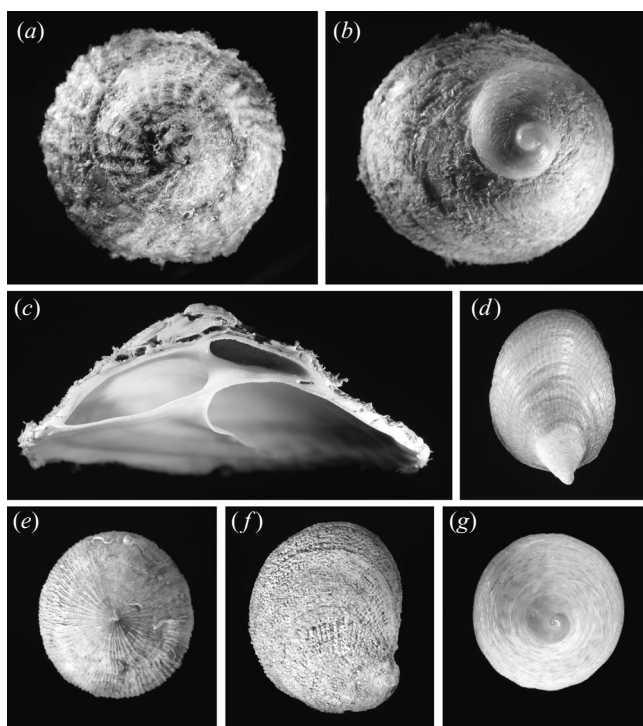


Figure 1. Examples of calyptraeid shells demonstrating coiled and uncoiled morphologies. (a) The coiled *Trochita calyptraeformis*. (b) The coiled *Sigapatella novaezealandica*. (c) Cross-section through *T. calyptraeformis* showing several whorls. Whorls near the apex have been filled with shell material. (d) The bilaterally symmetrical *Crepidula norrisiarum* is typical of the uncoiled species of *Crepidula*. (e) *Crucibulum radiatum* displays a conical morphology with no curvature or torsion. (f) *Bostrycapulus* cf. *aculeatus* from Panama are some of the most highly curved uncoiled calyptraeids. (g) *Calyptraea conica* displays a conical morphology with no curvature or torsion.

species with this coiled but limpet-like morphology (figure 1) are basal in the family (Hoagland 1977) and have yet to lose the ancestral coiling. The bilaterally symmetrical morphology of *Crepidula* is assumed to be a derived feature within the family.

(b) Quantification of shell coiling

We described and quantified the coiling of 184 post-larval shells of 46 species of calyptraeids by estimating three variables associated with coiling on the body whorl: the mathematical curvature of the growth trajectory of the aperture's centroid (Okamoto 1988, 1996), the arc-length of the coiled shell and the vertical direction of the coiling trajectory (figure 2). The mathematical curvature, the instantaneous rate of angular change of the vector tangent to the coil, is a measure of the number of degrees of lateral rotation undergone per unit length of the coil. This represents the tightness of the coiling. The arc-length of the coil represents not only the length of the coiled tube that makes up the shell, but, when measured in radians, the number of whorls or coils in the shell. Finally, the tangent angle (TA) of the coiling trajectory is a measure of the vertical translation along the coiling axis, and represents a measure of how high-spired the shell is (figure 2).

We used linear measurements taken from digital pictures of shells to estimate the curvature parameter. First, we estimated the axis of coiling by using either the 'projected axis' method of Schindel (1990) or by approximating its position and direction

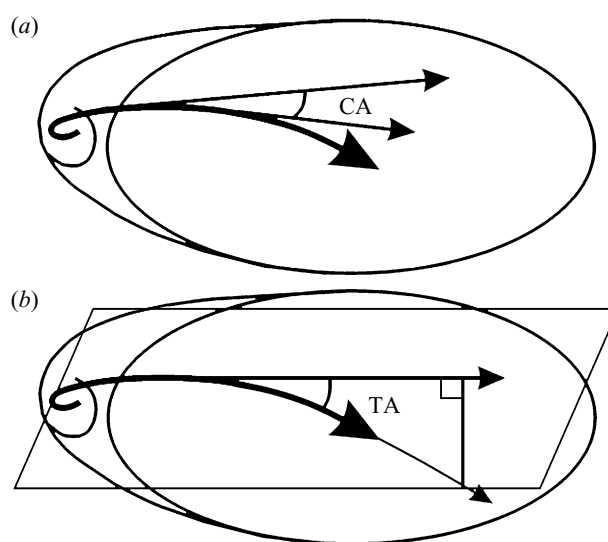


Figure 2. Variables used to estimate shell coiling parameters. (a) Curvature (κ) is the instantaneous rate of change of the cosine angle (CA) between tangent vectors along the growth trajectory (thick black arrow). (b) The tangent angle (TA) of the trajectory is measured between the plane perpendicular to the axis of coiling and the coiling trajectory. The arc-length of the growth trajectory is the length of the thick black arrow.

from a lateral view of the first teleoconch whorls, perpendicular to the shell's aperture. Two radii were measured from this axis to the centre of the shell tube. The positions of these two measurements on the coiled trajectory of the shell, measured in radians, were used to estimate the whorl expansion rate of Raup (W) (Raup 1961, 1966). Curvature (κ) was estimated from W , using (Cipriani 1999):

$$\kappa = \frac{1}{r \sqrt{((2.54 \times 10^{-2}) \times (\ln(W)))^2 + 1}}$$

where r is the radius at the position on the trajectory where W is estimated.

In a helicospiral isometric shell, the arc-length of each whorl is larger than the arc-length of the preceding whorl. Thus, the curvature is expected to decrease during shell growth. In isometric shells, the rate of change of $\log(\kappa)$ when estimated against the coiling trajectory measured in radians (slope of $\log(\kappa)$, or SLK), can be used to describe the isometric coiling trajectory of a shell (Cipriani 1999). It gives the rate of decrease of the tightness of the coil.

The TA of the trajectory was measured as the trigonometric tangent of the angle between the plane perpendicular to the axis of coiling and the tangent vector of the coiling trajectory. This gives an average measure of how much the shell coils downward and is related to the amount of translation along the coiling axis.

To discriminate coiled from uncoiled shells we created a morphospace using SLK, TA and arc-length expressed in radians. Linear measurements used to estimate these parameters were all standardized by the size (geometric mean of the height, width and length) of the whole shell. For reference, in figure 3, we included measurements of *Tegula verrucosa*, which has a shell similar to the classic example of a coiled gastropod shell (Raup 1966). Shells were coded as coiled or uncoiled for subsequent analyses on the basis of this morphospace.

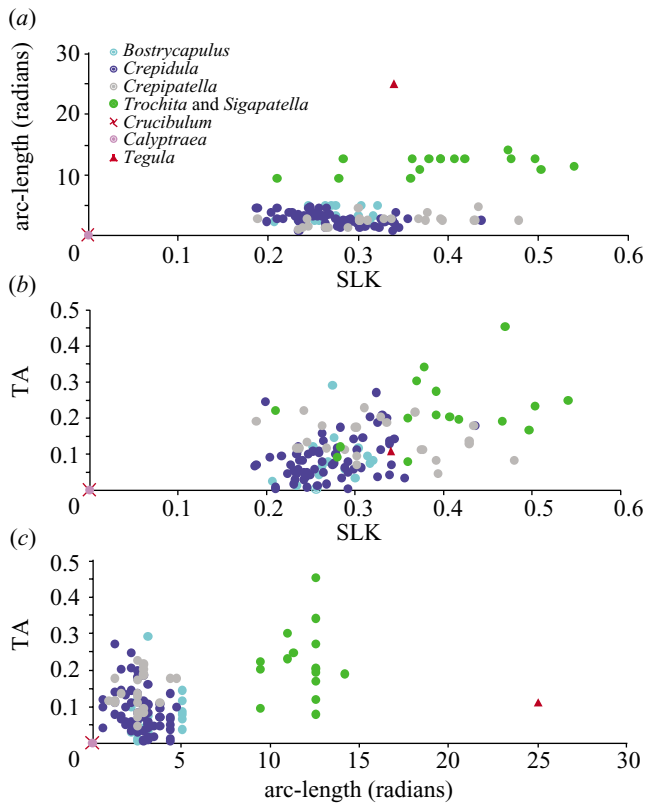


Figure 3. Shell coiling morphospace. (a) Plot of arc-length versus rate of change of curvature (SLK) for 184 calyptraeid shells. Points towards the right display a lower rate of decrease in curvature and points towards the top show higher levels of coiling. (b) Plot of the TA versus rate of change of curvature. Higher-spined, tightly coiled shells occur to the upper right. (c) Plot of TA versus arc-length. Again, more highly coiled shells fall towards the right. The six major shell morphotypes displayed by this family are indicated in the same colour as the respective species names in figure 4 (light blue dots, *Bostrycapulus*; dark blue dots, *Crepidula*; grey dots, *Crepidatella*; green dots, *Trochita* and *Sigapatella*; red cross, *Crucibulum*; pink dots, *Calyptraea*) and the red triangle represents *Tegula*, the classically coiled gastropod. Data points at the origin represent shells that are almost perfect cones and the single point on this graph represents 44 individuals of 10 species of *Calyptraea* and *Crucibulum* that show no coiling. *Tegula verrucosa* is included as a heuristic reference point as a representative of a ‘typical’ coiled shell with a familiar shape.

(c) **Phylogenetic and comparative analyses**

DNA sequence data from mitochondrial COI and 16S and nuclear 28S genes were used to create a phylogeny of 94 calyptraeid operational taxonomic units and a variety of outgroups (Collin 2002, 2003b). Both equal-weighted parsimony, matrix-weighted parsimony and Bayesian analyses resulted in similar topologies with high levels of Bayesian and non-parametric bootstrap support at many nodes (Collin 2003b) (figure 4). Most relevant for this study are the consistent and well-supported placement of *Sigapatella* and *Zegalerus* at the base of the tree, and placement of *Trochita* nested within *Crepidula*. The details and taxonomic implications of the phylogenetic analysis are dealt with elsewhere (see Collin 2003b).

The patterns of evolution of shell coiling were examined on the calyptraeid phylogeny in two ways. The states ‘coiled’ and ‘uncoiled’ were treated as unordered and mapped onto the

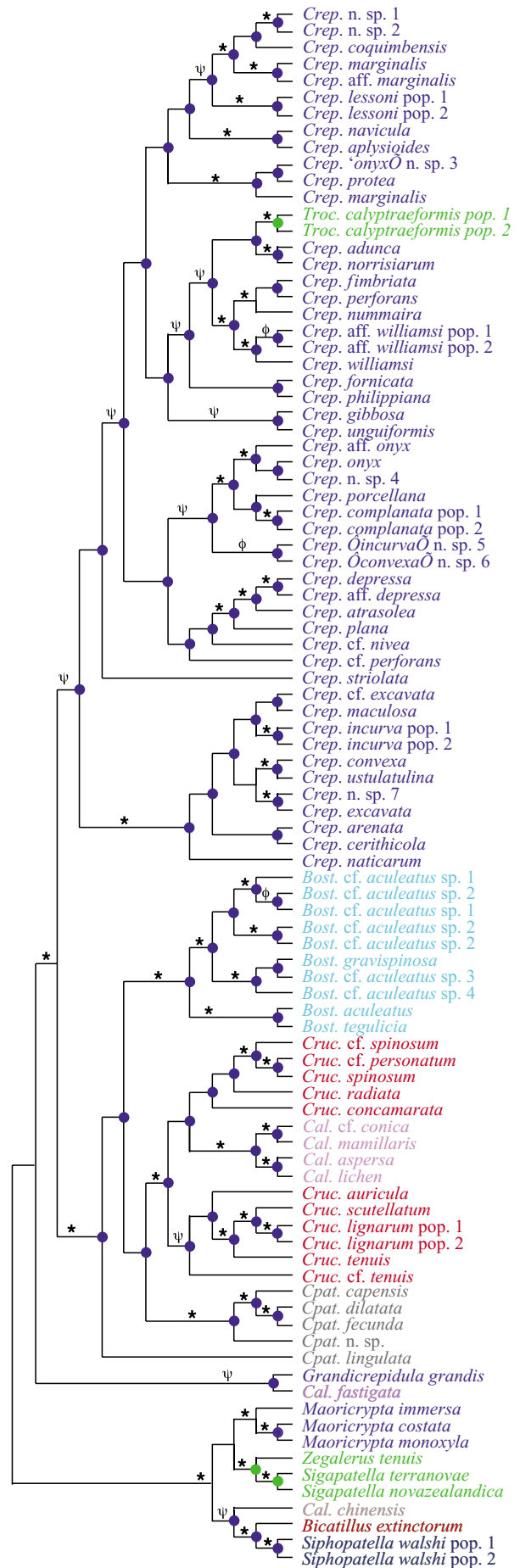


Figure 4. (Caption overleaf.)

Figure 4. Phylogeny of calyptraeids based on Bayesian analysis of molecular data. Values above branches indicate non-parametric bootstrap support greater than 70% (ϕ), Bayesian posterior probabilities of greater than 95% (ψ) and both types of support (*). In Bayesian, parsimony and weighted parsimony analyses, *Trochita* was always recovered as nested within *Crepidula* (Collin 2003b). Colours indicate the six overall shell morphotypes with only the taxa shown in green having clearly developed coiling. Coloured circles at the nodes indicate the maximum-likelihood reconstruction of the ancestral condition at that node as estimated by DISCRETE (Pagel 1999). Green, coiled, $p > 0.95$; blue, uncoiled, $p > 0.95$; no circle indicates that the node cannot be assigned to either state with statistical support. Crep, *Crepidula*; Cruc, *Crucibulum*; Bost, *Bostrycapulus*; Cpat, *Crepipatella*; Cal, *Calyptraea*; pop., population; n. sp., new species.

phylogeny using parsimony reconstructions as implemented by MACCLADE v. 4.0 (Maddison & Maddison 2000). Likelihood reconstructions of character evolution were performed using DISCRETE (Pagel 1999). The maximum-likelihood rates of character change were used to map the evolution of shell coiling onto the Bayesian topology using the 'calculate fossil likelihood' command. Those nodes where the likelihoods of the two states differed by more than two log units were considered to provide significant support for one state at that node in preference to the other state (Pagel 1999).

3. RESULTS

Our quantification of shell coiling shows that species of *Trochita*, *Sigapatella* and *Zegalerus* are significantly more coiled than any other calyptraeid species examined (figure 3). The high levels of coiling in the coiled calyptraeids, as well as the coiled exemplar *Tegula*, are almost entirely due to the larger number of whorls in the shell. Many calyptraeids, even the uncoiled ones, had values similar to those of the clearly coiled *Tegula* for the rate of reduction of coiling tightness (SLK) and the angle of coiling (TA). In fact, the coiled calyptraeids have higher levels of both coiling parameters than does *Tegula*. Although many calyptraeids have no measurable coiling at all (ca. 30% of the species examined here), several species display either a lateral or a dorsal-ventral curve in the shell. This curve in the shell results in the measurable values of SLK and TA, but the fact that these species do not complete a single rotation means that these values fail to create clear coiling. We coded species with shells that did not complete a single rotation as uncoiled in the subsequent analysis.

Mapping of shell morphology onto the phylogeny shows that there are two distinct clades of coiled calyptraeids, *Trochita* species nested within *Crepidula*, and a clade comprised of *Zegalerus* and *Sigapatella* towards the root of the tree. Equal-weighted parsimony unambiguously reconstructs two gains and a single loss of coiling if the ancestor of the family is reconstructed as coiled. If the ancestor is reconstructed as uncoiled, two gains of coiling and no losses are reconstructed. In both cases, coiling is reconstructed as having re-evolved in the ancestor of *Trochita*.

Maximum-likelihood reconstruction of ancestral states (Pagel 1999) reconstructs almost all of the nodes in the

tree as representing coiled ancestors (figure 4) with significant support. However, the states of the basal nodes in the tree are ambiguous. Because the closest outgroup of calyptraeids also contains a mixture of coiled and uncoiled taxa, addition of outgroups does not help to resolve the coiling condition at the base of the tree, and therefore likelihood analysis was performed without fixing the coiling condition at the root. However, it is clear that basal caenogastropods are coiled (Ponder & Lindberg 1997); therefore, coiling in *Trochita* represents a clear re-evolution of an ancestral state. It cannot be ruled out that coiling is also secondarily evolved in the clade including *Sigapatella* and *Zegalerus*.

4. DISCUSSION

Calculations of mutation rates of structural genes (Marshall *et al.* 1994) show that it is just possible that structures that have been lost for more than 10 Myr could re-evolve using the same genes. These estimates are based on the reactivation of a single silenced gene. Although the genetic basis of shell coiling is unknown, many morphological features are polygenic and therefore 6 Myr should be viewed as a generous estimate of the possible retention of genes underlying coiling (Marshall *et al.* 1994). The earliest convincing calyptraeid fossils date from the early Cretaceous and there are some possible internal moulds from the late Jurassic. The first incontrovertible *Crepidula* fossils appear in the late Cretaceous (99–65 Myr ago) and the genus was geographically widespread by the middle and late Eocene (49–33.7 Myr ago) (Hoagland 1977). A notable increase in diversity occurs in the early Miocene (23.8–20.5 Myr ago) when *Trochita* first appear (Taylor & Smythe 1985). Therefore palaeontological evidence suggests that the diversification of uncoiled *Crepidula* was well underway more than 10 Myr ago prior to the re-evolution of coiling in *Trochita* in the early Miocene.

The re-evolution of shell coiling after millions of years suggests that either (i) the genes that control shell coiling may have a separate function through which they maintained their integrity; or (ii) *Trochita* has evolved a different type of coiling from its coiled ancestor. The second hypothesis receives some support because the distinctly limpet-shaped coiling of *Trochita* superficially appears to differ from other coiled snails. The difference in shape is due primarily to the angling of the aperture towards the substrate, and to the growth of a skirt of shell material around the circumference of the shell. Detailed ontogenetic studies of the deposition of shell material would be necessary to determine whether the differences between coiling in *Trochita* and other coiled snails are superficially associated with the limpet form or if they represent a true difference in coiling geometry.

We prefer the hypothesis that genes and developmental pathways that underlie shell coiling have been maintained in *Trochita*'s uncoiled ancestors. Such pleiotropic maintenance of genetic components has been used as an explanation by authors reporting the few other cases of the re-evolution of complex characters. However, our explanation for the maintenance of shell coiling genes in

calyptraeids differs significantly from the previous examples. The other instances of the re-evolution of complex characters have all been explained by the use of genetic architecture and developmental pathways that have been maintained by expression in different places and/or structures in the organism (West-Eberhard 2003). These examples are almost all of meristic characters and include atavisms in whale hind limbs, toe number in horses and guinea pigs, number of molars in *Lynx* and wings of stick insects (West-Eberhard 2003; Whiting *et al.* 2003). Because the gastropod shell is not a meristic character, such heterotopic expression of genes for shell coiling cannot reasonably be proposed to account for our findings.

Instead we propose that heterochronic shifts in expression of the genes and developmental pathways that underlie shell coiling explain the re-evolution of coiling in *Trochita*. These pathways could have been retained if shell coiling was expressed in the larval shells of uncoiled calyptraeids. Neither *Crepidula adunca* or *C. norrisiarum*, the sister-species of *Trochita* recovered by our analysis, have free-living larvae with a coiled shell. In both species, direct development results in a juvenile with a bilaterally symmetrical shell (R. Collin, personal observation). In addition, the *C. fimbriata-williamsi* clade is also composed entirely of species with direct development (i.e. without a free-living larval stage). However, about half of the uncoiled calyptraeid species for which development is known have free-living planktonic larvae with clearly coiled shells (Collin 2003a). Ancestral reconstructions of development show that virtually any node in the tree *could* have had planktonic development (Collin 2002). Therefore, it seems likely that the genetic and developmental pathways for shell coiling have been retained in the larval stages of uncoiled calyptraeids and that coiling has evolved due to the heterochronic expression of these pathways in the adult shell of *Trochita*. If this is the case, we may expect to discover additional examples of the re-evolution of adult shell coiling in some of the many groups of uncoiled gastropods that produce larvae with coiled shells (such as capulids and hipponicids), but not in the two most species-rich clades of limpets, patellogastropods and fissurellids, whose larvae have virtually symmetrical cap-shaped shells. Although heterochrony has received wide attention for its role in morphological evolution and the generation of novelty, this is the first reported case, to our knowledge, of its role in the re-evolution of a complex character.

We thank C. Janis, D. Padilla and two anonymous reviewers for commenting on the manuscript. Sequencing was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution, with support from the Pritzker Foundation. DNA sequences are deposited in GenBank under accession numbers AF545871–AF545947 and AF550418–AF550453 (28S); AF545948–AF546016 and AF550454–AF550481 (16S); AF546017–AF546076 and AF550482–AF550514 (COI). Voucher material has been deposited at the Field Museum, Chicago, The Natural History Museum, London and The Academy of Natural Sciences, Philadelphia.

REFERENCES

- Cipriani, R. 1999 Construction, function, and evolution of accretionary morphologies with examples of larval and post-larval coiling in heterobranch gastropods. PhD thesis, University of Chicago, IL, USA.
- Collin, R. 2002 Evolution of mode of development in *Crepidula* (Gastropoda: Calyptraeidae): causes and consequences. PhD thesis, University of Chicago, IL, USA.
- Collin, R. 2003a Worldwide patterns of development in calyptraeid gastropods. *Mar. Ecol. Prog. Ser.* **247**, 103–122.
- Collin, R. 2003b Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of speciation. *Syst. Biol.* **52**, 618–640.
- Gould, S. J. 1970 Dollo on Dollo's Law: irreversibility and the status of evolutionary laws. *J. Hist. Biol.* **3**, 189–212.
- Gould, S. J. & Robinson, B. A. 1994 The promotion and prevention of recoiling in a maximally snail-like vermetid gastropod: a case study for the centenary of Dollo's Law. *Palaeobiology* **20**, 368–390.
- Hoagland, K. E. 1977 Systematic review of fossil and recent *Crepidula* and discussion of evolution of the Calyptraeidae. *Malacologia* **16**, 353–420.
- Kurtén, B. 1963 Return of a lost structure in the evolution of the felid dentition. *Comment. Biol.* **26**, 1–12.
- McLean, J. H. 1981 The Galapagos rift limpet *Neomphalus*: relevance to understanding the evolution of a major Palaeozoic–Mesozoic radiation. *Malacologia* **21**, 291–336.
- Maddison, D. R. & Maddison, W. P. 2000 *MACCLADE 4: analysis of phylogeny and character evolution*. Sunderland, MA: Sinauer.
- Marshall, C. R., Raff, E. C. & Raff, R. A. 1994 Dollo's Law and the death and resurrection of genes. *Proc. Natl Acad. Sci. USA* **91**, 12 283–12 287.
- Okamoto, T. 1988 Analysis of heteromorph ammonoids by differential geometry. *Palaeontology* **31**, 35–52.
- Okamoto, T. 1996 Theoretical modeling of ammonoid morphology. In *Ammonoid palaeobiology* (ed. N. H. Landman, K. Tanabe & R. A. Davis), pp. 225–251. New York: Plenum.
- Pagel, M. 1999 The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst. Biol.* **48**, 612–622.
- Ponder, W. F. & Lindberg, D. R. 1997 Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. *Zool. J. Linn. Soc.* **119**, 83–265.
- Raup, D. M. 1961 The geometry of coiling in gastropods. *Proc. Natl Acad. Sci. USA* **24**, 602–609.
- Raup, D. M. 1966 Geometric analysis of shell coiling: general problems. *J. Palaeontol.* **40**, 1178–1190.
- Schindel, D. E. 1990 Unoccupied morphospace and the coiled geometry of gastropods: architectural constraint or geometric covariation? In *Causes of evolution: a palaeontological perspective* (ed. R. M. Ross & W. D. Allmon), pp. 270–304. The University of Chicago Press.
- Taylor, J. D. & Smythe, K. R. 1985 A new species of *Trochita* (Gastropoda: Calyptraeidae) from Oman: a relict distribution and association with upwelling areas. *J. Conchology* **32**, 39–48.
- Vermeij, G. J. 1987 *Evolution and escalation*. Princeton University Press.
- Werdelin, L. 1987 Supernumerary teeth in *Lynx lynx* and the irreversibility of evolution. *J. Zool. Lond.* **211**, 259–266.
- West-Eberhard, M.-J. 2003 *Developmental plasticity and evolution*. New York: Oxford University Press.
- Whiting, M. F., Bradler, S. & Maxwell, T. 2003 Loss and recovery of wings in stick insects. *Nature* **421**, 264–267.