The origin of ascophoran bryozoans was historically contingent but likely

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The degree to which evolutionary outcomes are historically contingent remains unresolved, with studies at different levels of the biological hierarchy reaching different conclusions. Here we examine historical contingency in the origin of two evolutionary novelties in bryozoans, a phylum of colonial animals whose fossil record is as complete as that of any major group. In cheilostomes, the dominant living bryozoans, key innovations were the costal shield and ascus, which first appeared in the Cretaceous 85–95 Myr ago. We establish the parallel origin of these structures less than 12 Myr ago in an extant bryozoan genus, *Cauloramphus*, with transitional stages remarkably similar to those inferred for a Cretaceous clade. By one measure, long lag times in the first origins of costal shield and ascus suggest a high degree of historical contingency. This, however, does not equate with dependence on a narrow set of initial conditions or a low probability of evolution. More than one set of initial conditions may lead to an evolutionary outcome, and alternative sets are not entirely independent. We argue that, although historically contingent, the origin of ascus and costal shield was highly likely with sufficient possibilities afforded by time.

Keywords: Bryozoa; convergence; Cretaceous; evolutionary novelty; historical contingency

1. INTRODUCTION

Gould's (1989) thought experiment 'replaying life's tape' addressed the degree to which historical contingency shapes evolutionary outcomes. Results of the experiment could range from absolutely unpredictable, so that unique initial conditions will in every case produce highly different organismal phenotypes and interactions on this or any other world, to substantially replicable, with some evolutionary pathways favoured over others (Carroll *et al.* 2001; Vermeij 2006). In the latter case, we could expect familiar phenotypes and interactions to emerge despite differences in initial conditions, i.e. the range of genotypes and phenotypes inherent in, and environments experienced by, populations of a species at a particular point in time.

We cannot actually perform Gould's experiment at the scale he outlined, but the comparative method and experiments can provide relevant data that are otherwise rare (Gavrilets & Losos 2009). Convergent evolution in the broad sense (Arendt & Reznick 2007) is evidence that similar adaptations evolve from different sets of initial and subsequent conditions (Van Valen 1991; Conway Morris 2003). Vermeij (2006) analysed the distribution in the fossil record of both purportedly unique and convergent innovations and concluded the integration of previously existing components and selection channel living systems towards a replicable set of outcomes, thus

limiting the dependence of evolution on historical contingency. At a much simpler level, experiments by Blount *et al.* (2008) literally replayed the evolutionary tape. The origin of a Cit⁺ phenotype that allows *Escherichia coli* to use citrate as a carbon source in an oxic environment depended on earlier mutation(s) in the population, evidently demonstrating the importance of historical contingency in evolution.

Analyses involving the full history of life and those involving a few tens of thousands of bacterial generations differ considerably in scale. One could argue that the former lacks sufficient resolution at the mechanistic level, whereas the latter lacks sufficient complexity. Here we consider the degree of historical contingency in the origin of ascophoran cheilostome bryozoans, an example falling between these extremes.

Bryozoans are a phylum of colonial animals whose fossil record is as complete as that of any major group (McKinney & Jackson 1989). Cheilostomes, the dominant living group, appeared in the Late Jurassic. The earliest cheilostomes were of anascan grade, defined by zooids having a non-calcified frontal membrane. Anascans subsequently developed, around the frontal membrane, basally jointed spines that probably enhanced protection from small epizoic predators (McKinney *et al.* 2003; Lidgard 2008a). Calcification or loss of cuticular spine joints created rigid spines (costae), and fusions among costae led during the Late Cretaceous to the rigid frontal costal shield that defined a novel morphological grade, the cribrimorphs (Larwood 1969; Gordon 1994; Jablonski *et al.* 1997).

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Evolving within the large, paraphyletic anascan family Calloporidae (Gordon 2000), cribrimorphs diversified spectacularly (Ryland 1970), but today are greatly outnumbered by cheilostomes with other types of calcified frontal shield (gymnocystal, umbonuloid and lepralioid). However, the cribrimorph costal shield was a key innovation; prevailing evidence links gymnocystal and umbonuloid shields to cribrimorph ancestry, and lepralioid shields evolved from umbonuloid ones (Gordon & Voigt 1996; Gordon 2000). The costal shield in turn exposed a functional limitation. Muscular depression of the frontal membrane everts the feeding organ, or lophophore, through a hydrostatic mechanism. Reduction in the size of the costal shield and opesia (the area occupied by the frontal membrane) restricted this function. The origin beneath the shield of an associated novel structure, the ascus, or water compensation sac, circumvented the functional constraint (Voigt 1991; Gordon & Voigt 1996; Jablonski *et al.* 1997). Together, frontal shield and ascus define ascophoran-grade cheilostomes. These were non-trivial innovations. About 647 living and fossil genus-rank taxa have cribrimorph or other calcified frontal shields, whereas 404 are anascan, suggesting differential success of cribrimorphs and their descendants. Furthermore, the dichotomy between the anascan and ascophoran grades has pervaded higher level cheilostome classifications for the past century.

Cauloramphus is a morphologically well-defined calloporid genus comprising at least 25 living species; like the inferred ancestor of Cretaceous cribrimorphs, most species of *Cauloramphus* have basally jointed spines. Bryozoan collections from depths of 93–355 m in the Aleutian Islands, Alaska (Dick 2008), contained *Cauloramphus disjunctus* and two undescribed *Cauloramphus* species that show a series of increasingly derived cribrimorph morphologies culminating in an ascophoran-grade species. *Cauloramphus* is unknown in the fossil record before the Miocene (Duvergier 1924; Hayami 1970), which suggested independent origins of the costal shield and ascus in this genus. This provided a unique opportunity to investigate in a Neogene clade the evolutionary transitions leading to novelties that first appeared in a Cretaceous clade 80–90 Myr previously. To confirm this remarkable convergence, we reconstructed *Cauloramphus* phylogeny using cytochrome oxidase subunit I (COI) sequences. The fossil record and a COI molecular phylogeny indicate that the Neogene and Cretaceous clades underwent parallel evolutionary trajectories, from a similar starting point.

2. MATERIAL AND METHODS

(a) Specimen identification and microscopy

Morphologically identified *Cauloramphus* specimens all showed the following diagnostic suite of characters: circum-opesial spines, basally jointed avicularia and orificial spines, uniporous septula and a reduced, kenozooidal oecium; we verified the latter two characters with a scanning electron microscope (SEM). We took digital images with an S-2380 SEM (Hitachi) and an SMZ1500 stereoscopic microscope system (Nikon) and adjusted image brightness and contrast with Adobe Photoshop CS v. 8.0. We are preparing another publication on the taxonomy of Aleutian *Cauloramphus* and

will in that paper we cross-reference museum accession numbers with the specimens figured herein.

(b) DNA amplification and sequencing

We extracted DNA from ethanol-preserved or air-dried fragments of single colonies as described previously (Dick & Mawatari 2005) and saved a voucher fragment from each colony (table S1 in the electronic supplementary material). We performed PCR amplifications in 15 µl volumes containing 1 or 2 µl of non-quantified extract as the template source, standard amounts of other reagents (Dick *et al.* 2003) including universal COI primers LCO1490 and HCO2198 (Folmer *et al.* 1994) and Ex Taq DNA polymerase and buffer (TaKaRa), for 40 cycles at an annealing temperature of 40–45°C. PCR products were cloned and sequenced (Dick *et al.* 2003; Dick & Mawatari 2005). Sequences we obtained are available under GenBank accession numbers EU560977 and EU835947–EU835962.

(c) Phylogeny reconstruction and estimates of divergence times

We checked and aligned nucleotide sequences with ATGC v. 4.0.6 (Genetyx); the alignment was 658 bp long, contained no gaps and included 343 constant sites, 37 parsimony-uninformative sites and 278 parsimony-informative sites. We also constructed an alignment of deduced amino acid sequences with DAMBE (Xia & Xie 2001) that was 218 residues long (166 constant, 15 parsimony uninformative and 37 parsimony informative). We reconstructed phylogeny from nucleotide sequences using maximum parsimony (MP), neighbour joining (NJ) and maximum likelihood (ML) (Swofford 2000) and Bayesian analysis (BA) (Ronquist & Huelsenbeck 2003) and from amino acid sequences using MP and NJ. Plots of transitions and transversions against genetic distance with DAMBE showed that third-codon positions were highly saturated, so for MP analyses, we explored differential character weighting. MP analyses consisted of heuristic searches of 1000 random-addition replicates; we estimated bootstrap support for nodes through analysis of 10 random-addition heuristic searches for each of 100 pseudo-replicate datasets sampled from parsimony-informative characters only, with replacement. We constructed NJ trees based on log-determinant (LogDet) distances for the DNA dataset and mean character differences for the amino acid dataset; we determined bootstrap values by analysis of 1000 pseudo-replicates, with replacement. For ML and BA, the nucleotide substitution model determined to best fit (Posada & Crandall 2001) the data was GTR+Γ+I. The BA ran for six million generations, with trees sampled every 100 generations; from the resulting 60 001 trees, we discarded the first 15 000 as burn-in. We based estimates of divergence times on a Kimura-2 parameter (K2P) divergence rate of 1.6 per cent Myr⁻¹, the average of five COI rate values for marine invertebrates (Knowlton & Weight 1998; Schubart *et al.* 1998; Lessios *et al.* 2001; Marko 2002; Morrison *et al.* 2004).

3. RESULTS

(a) Morphology

We focused on four *Cauloramphus* species from the Aleutian Islands, only one of which has been formally described: CAC (*Cauloramphus* Aleutian sp. C), DIS (*C. disjunctus* Ortman, 1890), ALA (Aleutian sp. A) and

Figure 1. Morphology of zooids of inferred primitive and derived (cribrimorph) *Cauloramphus* species. (a) CAC. (b) DIS (*C. disjunctus*). (c) DIS, fossil, Lower Pleistocene, Kuromatsunai, Hokkaido, Japan. (d) ALA. (e) CLC. (f) CLC (bleached), medially interlocking ends of costae. (g) CLC (zooid interior, bleached), entrances to spine lumens (black arrows), parietal muscle scars (white arrows) and opercular condyles (white arrowheads). (h) CLC (light micrograph, inverted image), outlines of ascus (black arrows) and opesia (white arrowheads). (i) CLC, partially open operculum and suboral opening to ascus (arrow). Unless otherwise noted, specimens were dried, unbleached. All except (h) are SEM images. Scale bars, 100 µm (a–e, g, h), 25 µm (f) and 50 µm (i).

CLC (cribrimorph-like *Cauloramphus*). CAC (figure 1a) is stereotypical for the genus, having flexibly jointed spines and an exposed frontal membrane. The others show increasingly derived cribrimorph morphologies.

In DIS, the spines angle over the frontal membrane to form a tight cage, but are not fused laterally or medially (figure 1b). These spines have the basal joint calcified in mature zooids and are thus costae, by definition (Gordon 2000). A Pleistocene fossil that retains the spines confirms the loss of the cuticular joint (figure 1c).

ALA has a frontal shield formed by slightly flattened, weakly fused costae (figure 1d); as with DIS, the spine joints are calcified. The frontal membrane and operculum are unmodified from the stereotypical condition (figure 1a). The opesia is scarcely reduced, lies well below the costal shield and is completely accessible to water flow via the distal opening and small basal slits between costae.

CLC has derived costae (figure 1e) interlocking in the mid-line, with subterminal expansions forming a suture with expansions from adjacent costae (figure 1f). The area of the opesia has become so reduced (figure 1g,h) that depression of the frontal membrane alone cannot provide sufficient volume change to effect lophophore eversion. In this species, an ascus extends beneath the sloping walls surrounding the opesia (figure 1h). The operculum is much thicker than the membrane proximal to it and tightly fits a skeletally defined orifice (figure 1e). During lophophore eversion, water enters the ascus through tiny slits between the costal bases and a suborificial opening (figure 1i) created as the operculum opens; weak condyles (figure 1g) provide a fulcrum for tilting

the operculum. With the well-defined orifice, frontal shield and ascus, CLC has unambiguously achieved ascophoran grade.

(b) Phylogeny and divergence times

Phylogenetic analyses of a 658 bp fragment of the mitochondrial COI gene by four methods (NJ, MP, ML and BA) all showed the cribrimorph taxa to be well embedded within *Cauloramphus* (clade A), with high-support values for this clade (figure 2). The average pairwise K2P distance between clades B and C was 24.55 per cent, providing an estimate of 15.3 Ma (early Middle Miocene) for the earliest divergence in the genus. This corroborated the Miocene origin of *Cauloramphus* inferred from fossils from Japan (putative *Cauloramphus* from the Langhian-Serravallian, Middle Miocene; Hayami 1970) and France (Tortonian, Late Miocene; Duvergier 1924).

All methods produced trees with a similar backbone topology (asterisks in figure 2 indicate nodes in common), but which otherwise showed either (i) the four species in clade F forming a monophyletic group, with either of two different internal arrangements, or (ii) the four species in clade F comprising a paraphyletic group basal to clade E (figures S1–S4 in the electronic supplementary material). Figure 2 shows the cribrimorph taxa (clade G) as monophyletic, with CAC as the sister group; this hypothesis is the best supported by morphology. Three morphological synapomorphies (calcification of the cuticular spine joints, loss of granulated cryptocyst and presence of obligately paired frontal

Figure 2. NJ tree based on the analysis of a 658 bp fragment of the COI gene using LogDet distances, with third-codon positions omitted. Asterisks indicate nodes congruent in optimal trees from four methods of analysis; bootstrap values above branches are from analysis of 1000 pseudo-replicates. *Cauloramphus* species names: COA, Oshoro sp. A; KVA, Korean sp. A; SPI, *C. spinifer*; CAR, *C. cryptoarmatus*; MUL, *C. multiavicularia*; KOR, *C. korensis*; CLC, cribrimorph-like *Cauloramphus*; ALA, Aleutian sp. A; DIS, *C. disjunctus*; CAC, Aleutian sp. C; MSP, *C. multispinosus*; CIN, *C. intermedius*; MAG, *C. magnus*; CNI, *C. niger*; COB, Oshoro sp. B. Outgroup names: ARM, *Tegella armifera*; ARC, *T. arctica*; BUG, *Bugula neritina*.

avicularia) support clade G, whereas alternative topologies all require either two independent gains of the cribrimorph condition or a gain followed by reversion to the plesiomorphic condition.

Figure 3 illustrates the evolutionary transition series from a stereotypical *Cauloramphus* species (common ancestor of CAC and clade G; figure 3a), to DIS with the opesia spine bases calcified (figure 3b), to the cribrimorph common ancestor of ALA and CLC (figure 3c) and to ascophoran-grade CLC (figure 3d). This transition was rapid; average pairwise K2P distances between CAC and clade G (18.25%) and between ALA and CLC (12.12%) indicated that not more than 3.8 Myr elapsed between initial calcification of the spine bases (after divergence of clade G from CAC; 11.4 Ma) and attainment of a conspicuously cribrimorph morphology (common ancestor of ALA and CLC; 7.6 Ma). Although *C. disjunctus* (DIS) occurs in the shelf zone from the western Aleutian Islands, Alaska, to the Sea of Japan, ALA and CLC are unknown outside the Aleutians, suggesting that the cribrimorph *Cauloramphus* clade evolved in the Miocene in the western Aleutians. Dick (2008) discussed the possibility of a similar Aleutian radiation in the cheilostome genus *Monoporella*.

Figure 3. Stepwise morphological stages in the transition from a spiny anascan to an ascophoran cribrimorph. Dark grey stippling, frontal membrane (i.e. non-calcified frontal wall); light grey shading, coelomic cavity. (a) Stereotypical ancestral anascan with basally jointed spines surrounding the frontal membrane, like CAC. (b) Species with independent costae angled over the unmodified frontal membrane, like DIS. (c) Species with costae weakly fused to form a continuous, rigid shield over the unmodified frontal membrane, and with the area of the opesia not reduced, like ALA. (d) Species with the costal shield reduced in area and overlying an ascus, like CLC. Dark grey stippling indicates the part of the original frontal membrane that we infer is incorporated into the floor of the ascus; the extracostal portion of the ascus (white) is labelled.

4. DISCUSSION

(a) Parallel evolutionary transitions in Neogene and Cretaceous lineages

The evolutionary transitions of the cribrimorph *Cauloramphus* species parallel those inferred in the cribrimorph radiation (Larwood 1962, 1969, 1985), both starting from spiny calloporid ancestors. Each of the cribrimorph *Cauloramphus* species has morphological analogues among Cretaceous cribrimorphs. DIS is analogous to the earliest cribrimorphs, the myagromorphs, with rigid open cages of individual costae. ALA, with distinct costae not tightly fused laterally, resembles species of Late Cretaceous *Leptocheilopora*. In CLC, the costae remain distinct, though with subterminal lateral expansions such as those in some species of Cretaceous *Pelmatopora*. The ascus in CLC is equivalent to that inferred in the Late Cretaceous cribrimorph *Pliophloeia* and cribrimorph-derived ascophoran *Boreasina* (Voigt 1991; Gordon 2000), taxa that also have well-defined, ascophoran-like orifices.

Unlike most ascophorans, which have only a single suborificial opening leading into the ascus, CLC has multiple tiny intercostal openings in addition to the main,

suborificial opening. CLC represents an intermediate stage seen in other transitions from cribrimorphs to ascophorans with a solid shield. A Late Cretaceous ascophoran ('undescribed genus and species') reported by Gordon & Voigt (1996) likewise has both an ascophoran-like orifice and vestigial intercostal slits. In Late Cretaceous *Systemostoma asperulum* and *Platygena* sp. (Gordon & Voigt 1996), the costal shield is reduced to a tiny area just proximal to the orifice and is functionally equivalent to the single suborificial opening of most ascophorans.

Calcification of the opesial spine joints is an initial stage common to the evolutionary trajectories in both the Cretaceous and the Neogene lineages, as both DIS and myagromorphs (Larwood 1985) are basal to taxa with fully formed costal shields. The mechanism behind loss of the spine joints is unknown, but simple possibilities include selection on existing variation in joint size, heterochrony in calcium deposition during spine growth or mutation in a gene regulating calcium deposition. Once spines become rigid, they become more susceptible to breakage. Simultaneous selection for reinforced costae and frontal protection would favour a flatter arch of the costae over the frontal membrane, distal and lateral fusions among costae and reduction in area of the costal shield, all of which presumably involve readily selectable shape changes in simple structural traits.

Origination of an ascus is the ultimate stage in the evolutionary transitions. In CLC, developing zooids first form the costal shield and reduced frontal membrane, after which the ascus develops rapidly. We infer that this occurs by delamination of the frontal epithelium from the inner skeletal wall and secretion of new cuticle to form the periphery of the ascus floor, which also includes the frontal membrane, an interpretation consistent with previous descriptions of ascus development (Harmer 1902; Tavenner-Smith & Williams 1970). This process would permit a gradual evolutionary increase in the size of the extracostal ascus in lineages undergoing a reduction in the costal shield.

(b) Forces influencing the parallel evolutionary trajectories

Two forces appear to have driven similar trajectories in Cretaceous and the Neogene cribrimorph clades. One is continuous, diffuse selection favouring frontal protection, primarily in the form of a physical barrier to small nudibranch gastropods, amphipods, copepods, pycnogonids, errant polychaetes, nematodes and others (Jackson & McKinney 1990; McKinney *et al.* 2003; Lidgard 2008b). Many living epibiont predators preferentially attack still-uncalcified zooid buds or attempt to circumvent the frontal shield, attacking an everted polypide or the zooidal operculum. Induction of spine formation and triggered onset of colony reproduction are known from experimental predation studies. Regeneration of damaged individual zooids is seen from the Early Cretaceous onwards. Consumption of tissue, regeneration, frontal shield or spine formation and resource allocation to larva production all extract metabolic costs from colonies, with implied fitness consequences. Additional indirect evidence comes from the inference of other cases of the independent evolution of a costal shield in cheilostomes. Examples include *Aspidelectra* (Electridae),

a malacostegan genus distinct from the lineage(s) that gave rise to the Cretaceous cribrimorphs, and *Callopora rylandi*, *Membraniporella nitida* and *Valdemunitella spinea*, all attributed to the Calloporidae (Gordon 1986; Hayward & Ryland 1998).

The other force driving convergent evolutionary trajectories is the functional constraint to retain the hydrostatic lophophore-eversion mechanism. While the ascus itself provides no selective advantage against predation, it is nonetheless indirectly under selection by predation. Whenever selection for a stronger, thicker frontal surface involved a gradual reduction in the area of the costal shield (and hence of the frontal membrane), there would have been concomitant selection for the origin and expansion of an extracostal ascus, in order to maintain sufficient volume for lophophore eversion. Inability to achieve an extracostal ascus would effectively inhibit a reduction of the costal shield past a functional threshold. Not all lineages that evolved a costal shield overcame this constraint; for instance, none of the preceding examples has achieved ascophoran-grade morphology. However, like *Cauloramphus*, families Cribrilnidae, Cate nicellidae and Petalostegidae contain some living species with a complete costal shield overlying an unreduced frontal membrane (as in figure 1d) and others with a reduced or vestigial costal shield and extracostal ascus (as in figure 1e) (Harmer 1902, 1926; Banta & Wass 1979; Gordon & Hondt 1991; Gordon 2000), indicating the independent origin of an ascus in each family.

We can document the completely independent origin of costal shield plus ascus in only two clades, *Cauloramphus* and a Cretaceous cribrimorph clade, as it is unclear whether any of the families in which the ascus evolved independently also originated the costal shield independently. A comprehensive molecular phylogeny of cheilostomes will be necessary to clarify this issue.

(c) The role of modularity

The likelihood of cheilostomes evolving a rigid frontal shield from calloporid ancestors would have been higher if there were ways to evolve a shield in addition to fusion of costae. Gordon (2000) evaluated a number of models for the evolution of ascophoran shields, some of which do not require cribrimorph intermediates (e.g. gymnocystal and cryptocystal shields). The fossil record, however, overwhelmingly supports the conclusion that most ascophoran shields can be traced through cribrimorph intermediates (Voigt & Gordon 1995; Gordon & Voigt 1996; Gordon 2000), which may indicate that there are constraints on other modes. That costae were favoured as the route to increased frontal protection underscores the importance of the union, cooperation and integration of previously independent components in the origin of evolutionary novelties (Vermeij 2006). The brood chamber is another key cheilostome novelty (Taylor 1988) that originated from articulated spines and is inferred to have evolved independently through a parallel transition series in more than one clade (Ostrovsky & Taylor 2005).

(d) How contingent were the origin of costal shield and ascus?

In their bacterial experiment on the role of historical contingency in evolution, Blount *et al.* (2008) grew *E. coli*

in an oxic environment in a glucose-limited medium also containing citrate; *E. coli* cannot use citrate in an oxic environment. They observed no evolution of the capacity to use citrate for more than 30 000 generations, but a variant (Cit⁺) with this capability evolved in one population by 31 500 generations. They were able to demonstrate that a 'potentiating mutation' or mutations had arisen by 20 000 generations, creating a genetic background in which the Cit⁺ variant was more likely to evolve subsequently—in other words, in which parallel evolution was likely to occur.

The costal shield and ascus in cheilostomes also showed long delays in origination, followed by multiple, parallel origins. Articulated lateral and proximal peripodial spines first appeared in a species of *Spincharixia*, of Aptian age, 125–112 Ma (Taylor 1986); the myagromorphs, with non-articulated but independent spines, appeared early in the Cenomanian, approximately 97–94 Ma (Larwood 1985); and the ascus is inferred to have first appeared in a cribrimorph lineage by the Coniacian, approximately 89–86 Ma (Gordon 2000). Assuming a mid-Aptian age for the origin of articulated spines, at least 22 Myr elapsed between the origin of articulated spines and the origin of the costal shield and from there up to approximately 11 Myr before the origin of the ascus. These delays could represent the times required for the accumulation of mutations creating potentiating genetic backgrounds, as occurred in *E. coli*.

Another factor contributing to the long delays in the origin of the costal shield could have been the intrinsically low speciation rate of primitive planktotrophic cheilostomes posited by Taylor (1988). In addition, selection regimes broadly experienced by Cretaceous bryozoans could have changed or intensified in the Cenomanian in ways that influenced the origin of the costal shield, subsequent reduction of the shield and origin of the ascus. Although marine predation increased through the Mesozoic (Vermeij 1977), zooid-scale skeletal armament would not have deterred the associated macro-predators, but would have deterred small epibiont predators, which are the dominant consumers of living bryozoans (Lidgard 2008a,b). That early anascans originated articulated spines in the first place, presumably also in response to selection by predators, argues that predation was a continuous, pervasive factor and that potentiation delays were involved in the origins of costal shield and ascus.

Foote (1998) suggested assessing the degree of contingency of a particular biological property by comparing the amount of time that environmental conditions have been favourable for the origin of the property with the amount of time the property has actually existed: the longer the lag time in the origin of the property, the more historically contingent it is. By this measure, the origins of the costal shield and ascus, with long lag times, were highly historically contingent. Lag time, however, may not be an appropriate measure of contingency. Assuming that accumulation of a particular set of mutations that result in a potentiating genetic background is a random process, we can expect lag times to have a Poisson distribution; sometimes the potentiating background will arise rapidly, sometimes slowly.

Defining historical contingency to mean that an evolutionary outcome depends on one particular set of initial conditions is too narrow. Although a particular

history is actualized in a particular evolutionary outcome, it is probably not the only history that could have done so. Studies investigating the molecular bases of convergent traits have shown that there can be multiple routes to a particular solution (Prud'homme *et al.* 2006; Arendt & Reznick 2007; Wray 2007). Furthermore, although a particular set of historically contingent conditions will include a selective force as part of the environment, generalized selective forces such as predation can be continuous over long periods of time, meaning that permissive sets of initial conditions are not totally independent.

We return to the question of how contingent on history was the origin of the costal shield, once anascans with articulated frontal spines had evolved (a similar consideration applies to the origin of the ascus). We argue that the origin was highly contingent on a set of initial conditions, but that given time, a genetic background would arise (analogous to flipping a coin long enough to achieve 10 heads or tails in a row) that was visible to the continuous, diffuse selective force of predation, opening the way for an adaptive solution. To evolve a variant capable of using citrate, *E. coli* took only 30 000 generations, a pittance in geological time. Assuming one generation per year, cheilostome bryozoans took at least 22 million generations to achieve the first costal shield, but ultimately they got the job done.

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