The Openness-Constraint Spectrum: Module Integrity and the Evolutionary Reversibility of Fungal Traits

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Abstract

Fungal traits differ sharply in whether evolution can retrace its steps. Hymenophore morphologies transition bidirectionally across phylogenies, whereas gasteroid fruiting bodies, arbuscular mycorrhizal symbioses, and obligate parasitism show little or no macroevolutionary return. We propose module integrity, the preservation state of a gene regulatory module relative to its ancestral baseline, as a unified variable, measurable in principle, that predicts where a trait falls on this reversibility spectrum. Integrity erodes when a non-compensable bottleneck core, a top-level regulatory node with no substitute, is lost: downstream coordination collapses, and once degradation passes a critical zone, reversal becomes statistically inaccessible. The resulting constraint is trait-specific; a lineage locked in one trait continues to explore in others. A parallel diversity-generating pathway, namely variation supply, quasi-neutral retention, somatic expansion, and selective recycling sets how widely a lineage explores, and module integrity sets what it can return to. The two meet at the quasi-neutral plateau, which serves both as the

medium of exploration and as the entry channel of erosion. Module integrity has not yet been measured. We specify how to estimate it from gene content, regulatory topology, and expression response; the association test that would support or refute the core claim; and the decay-sequence prediction that separates this account from gene-count alternatives. Drift, weak selection, adaptation, and horizontal gene transfer each enter the causal narrative at a specified point.

Key words: module integrity; evolutionary reversibility; bottleneck core; developmental constraints; evolvability; gene regulatory networks; quasi-neutral fitness plateau; fungal macroevolution

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I. Introduction: the coexistence of openness and constraint

The fungal kingdom presents a striking macro-pattern whose full shape becomes visible only when observations from different scales are placed side by side.

At the morphological level, Agaricomycetes display a clear contrast. Conversions among complex multicellular structures (gills, pores, teeth, and other hymenophoral types) occur frequently and bidirectionally across phylogenetic trees, as do many

macroscopic configurations of fruiting bodies. Gasteroid morphology, a closed form in which spores mature inside the fruiting body, follows a different logic.

It has originated independently more than a hundred times within Agaricomycetes, in lineages scattered across major clades and occupying habitats from arid grasslands to tropical cloud forests (Hibbett et al., 1997; Hibbett, 2004; Varga et al., 2019; Sánchez-García et al., 2020); no clear case of return to an open, ballistosporic form has been reported. This asymmetry between gain and loss corresponds neither to the morphological complexity of the transitions nor to the ecology of the habitats involved.

A parallel asymmetry appears in nutritional ecology.

Orchid and ericoid mycorrhizae are generally considered broadly reversible (Martino et al., 2018; Adamo et al., 2020), and ectomycorrhizal symbiosis has arisen repeatedly from saprotrophic ancestors, with reversions to free-living lifestyles inferred in some lineages (Hibbett, Gilbert & Donoghue, 2000; Kohler et al., 2015; Martin et al., 2016).

Arbuscular mycorrhizal fungi, once obligately symbiotic, appear to leave no clear path back (Miyauchi et al., 2020; Supplementary Box 2, case 4). Saprotrophic generalists have moved toward specialization independently many times, and the likelihood of reversal varies among symbiosis types: some lineages retain the capacity to revert, whereas others appear locked. Reversibility thus approximates a spectrum whose position is not set simply by the "depth" of the symbiosis.

The same duality recurs at the molecular scale. Biosynthetic gene clusters underlying fungal secondary metabolites show remarkable diversity, evidence that evolution continually explores novel chemical functions (Rokas, Wisecaver & Lind, 2018). Yet the landscape also holds functionally redundant clusters, like different architectures producing the same or highly similar products, and pseudogenized or fragmented relics

(Throckmorton et al., 2016; Ozturk et al., 2019). The adaptive significance of many secondary metabolites remains unclear over long periods, and their persistence is which isn't explained completely.

At the species scale, many fungal genera contain species complexes whose member lineages are already strongly differentiated genetically, coexist sympatrically, and yet are nearly indistinguishable morphologically (Taylor et al., 2000; Harder et al., 2013); a single genotype, conversely, can produce markedly different morphologies under different environmental conditions (Ekanayaka et al., 2025; Dan et al., 2026). This decoupling between genetic differentiation and phenotypic variation complicates taxonomy, and it indicates a distinctive property of how fungal developmental systems translate genetic variation into morphological output.

These observations may not be independent puzzles. Viewed together, they point to a common structural factor: a systematic decoupling between genetic change and its phenotypic or functional consequences, which lets some variation accumulate without decisive fitness effects and lets evolutionary states be explored. The decoupling is also asymmetric. From flexible free-living starting points, independent lineages have repeatedly moved toward morphologically self-limiting gasteroid forms, ecologically self-constraining obligate symbioses, and parasitism, while the return path has nearly closed on macroevolutionary timescales; many lineages, meanwhile, retain high morphological and ecological flexibility. Widespread free exploration alongside systematic closure of return paths suggests that the orientation of the decoupling is itself modulated by a structural factor independent of any particular ecological scenario: under some conditions, decoupling permits or even promotes exploration; under others, it closes off return.

Existing theoretical resources each illuminate part of this picture. Phylogenetic comparative methods quantify transition-rate asymmetries but do not explain why some transitions are reversible and others are not. Population-genetic theory specifies when drift or selection dominates, but has modeled trait loss largely as the degradation of independent genes. The molecular evolution of gene loss explains why individual degraded genes are rarely restored. Multiple independent reversions would be required, but treats restoration as the accumulation of independent mutational events, not as the reconstruction of lost regulatory coordination (Marshall, Raff & Raff, 1994; Albalat & Cañestro, 2016). The developmental-constraints tradition has long asked why some variants are never produced (Maynard Smith et al., 1985); Dollo's law names irreversibility without supplying a mechanism (Gould, 1970; Elmer & Clobert, 2024). Two components of the explanation have received less attention: a continuous variable that predicts where a transition falls on the reversibility spectrum, and an architectural criterion that separates reversible from irreversible losses. Why, then, does the same group of organisms show striking evolutionary openness in some traits and pronounced directional constraint in others, and why does the decoupling between genetic variation and phenotypic consequences recur at every scale, from molecules to species? This question is not unique to fungi, but the fungal kingdom provides an unusually favorable system in which to pose it.

A reversal must pass through two stages in sequence. The developmental system must first regenerate the lost variant at a non-negligible rate; the regenerated variant must then survive selection and drift to fixation. The second stage presupposes the first: selection cannot return what development can no longer produce. One familiar explanation of irreversibility rests on the second stage that lineages do not re-evolve open fruiting bodies because nothing selects for them. It carries a tacit premise that each

lost trait remains regenerable at a comparable rate, and the reversibility spectrum is set wholly downstream, by trait-specific ecological and genetic circumstances. This premise is the null hypothesis. We propose that regeneration acts as a differential constraint across traits that are architectural in basis, measurable, and predictive of where a transition falls on the reversibility spectrum. If this upstream gate is real, the earliest relaxation of selection should localize to bottleneck cores and their regulatory connections (Expectation 3), and lineages locked in derived states should show coordinated degradation at those cores (Expectation 5). If the spectrum is set downstream, degradation should follow ecological release and be ordered by each gene's susceptibility, as determined by mutation target size, expression level, and pleiotropy, rather than by regulatory position.

The upstream variable is module integrity: the systemic preservation state of a gene regulatory module, relative to its ancestral baseline, expected to govern where a transition falls on the reversibility spectrum. It addresses the reversibility dimension specifically. Drift, weak selection, adaptation, and horizontal gene transfer continue to shape the macroevolutionary landscape of fungi, and the framework specifies where each enters the causal narrative. Integrity should operate across a wide range of fungal groups, though its magnitude and molecular realization differ among lineages. A second, parallel dimension of the same evolutionary process is exploration breadth, set by a four-stage diversity-generating pathway whose determinants are distinct from those that maintain module integrity.

We advance that reversibility, so defined, is governed by the preservation state of the gene regulatory module that encoded the lost trait. This preservation state, or module integrity, erodes decisively when non-compensable bottleneck cores are lost. The account, however, remains untested: its central variable is unmeasured. Although the

framework is developed here for fungi, it is intended to specify a candidate general dimension of reversibility and a complementary perspective on macroevolutionary process more broadly. In this sense, the distinctive fungal landscape serves both as a demanding test case and as a route from a special case toward a more unified picture. This paper therefore specifies that variable, its measurement, and the tests that would confirm or refute the account. Sections II and IV develop the variable and its erosion; Section III, the diversity-generating pathway; Section V, the expectations and their designs; and Section VI, the scope and order of the research programme.

II. Module integrity and bottleneck cores

A gene regulatory module (GRM) is the network that encodes and executes a specific biological function or developmental process. Module integrity refers to the structural and functional state of such a module as maintained over evolutionary time. The comparisons discussed here apply to orthologous modules confirmed by phylogenetic evidence: regulatory network structures inherited from a common ancestor through vertical descent. The module concept corresponds to the functional subcircuits of hierarchical gene regulatory network theory, in which developmental systems are organized into nested regulatory units with distinct topological positions and evolutionary dynamics (E. H. Davidson & Erwin, 2006; E. H. Davidson, 2010; Erwin & Davidson, 2009). Non-homologous modules performing similar functions fall outside the standard scope, because no common and reliable integrity reference can be established for them.

Module integrity is continuous. Values near the intact state indicate no significant departure from the ancestral baseline; values near zero indicate deep deconstruction or

functional loss. The intact state is the predicted structure and expression baseline of the module at the most recent common ancestor (MRCA) of the lineages under discussion, inferred by ancestral state reconstruction (Meade & Pagel, 2022). Inference quality depends on the phylogenetic framework and the density of taxon sampling: deep divergence and sparse sampling widen the confidence intervals of inferred ancestral nodes. This bound applies to every cross-lineage claim made below. Integrity is thus an inferred quantity. It is defined as the cumulative departure of an independent lineage from the ancestral node under the combined effects of genome degradation, phylogenetic age, and gene functional constraints. Its operational implementation is part of the research agenda (Supplementary Box 1).

Integrity values are comparable only within a homologous framework. Lineages measured against different reference states or on different scales are not directly comparable. The ideal comparison objects share the same ancestral node, diverged relatively recently, and maintain clear orthologous relationships; a unified baseline then supports direct comparison among members. Where no unified homologous framework can be established, integrity can still be inferred within individual lineages, but cross-lineage numerical comparisons require caution. The phylogenetic structure and data availability of a study system set the upper limit of interpretability; widened confidence intervals on inferred ancestral nodes must be reported explicitly, and sample selection is essential for ensuring comparability.

An alternative formulation, with roots in the Dollo's law literature, attributes irreversibility to the number of genes requiring restoration: multiple degraded genes would all need to be rebuilt, and the joint probability is low (Marshall et al., 1994; Collin & Miglietta, 2008). The logic is sound as far as it goes. It treats each gene as an independent restoration problem and does not capture the architectural dimension:

when a bottleneck core is lost, the regulatory connections through which downstream genes were coordinated must themselves be rebuilt, and the restoration problem changes in character. A top-level node controlling hundreds of downstream targets may be encoded by one or a few genes; retention of many non-bottleneck genes is not sufficient for the integrity of the regulatory architecture. Gene number alone does not distinguish a module that is intact but missing a few effectors from one whose coordination has been dismantled. The core measure of module integrity is the topological state and dynamic response capacity of the regulatory architecture, operationalized through genic content, network topology, and expression response (Supplementary Box 1). Throughout the paper, module integrity denotes this construct; attractor-landscape and neutral-network vocabulary, introduced where needed, is expository for it rather than a second definition. The premise that regulatory architecture, rather than gene inventory, determines the constraints on evolutionary trajectories has been developed independently in the systems-biology literature, where mechanistic models of gene regulatory networks have been used to predict evolutionary constraint at multiple levels of network organization (Nghe et al., 2020). The present framework shares that premise but shifts the question: not which evolutionary step is next, but whether a lost trait can be regained. That shift requires treating the regulatory architecture as a variable whose preservation state changes over macroevolutionary time.

The two accounts diverge on a specific configuration: a trait lost through inactivation of a single non-compensable core while dozens of downstream genes remain. The gene-count account predicts facile reversal, since few genes need rebuilding; the architectural account predicts closure, since coordination, not genes, is what was lost. The *Schizosaccharomyces* pair (Section IV(2)) and the recurrent loss of *abaA* (Expectation

5) approximate this configuration and are its natural first tests.

Module integrity stands in explicit relation to the classical concept of developmental constraints, which refers to biases in the production of variant phenotypes caused by the structure, composition, and dynamics of the developmental system (Maynard Smith et al., 1985).That tradition concerns evolutionary accessibility: the state of the developmental system conditions where evolution can readily go. Its attention has fallen mainly on forward accessibility why certain variants are never produced. Module integrity addresses the complementary direction: return accessibility why certain variants, once produced and then lost, cannot readily be produced again. Both questions belong to one paradigm and differ only in the direction of accessibility they measure. Developmental constraints concern how developmental architecture biases the production of variation. Module integrity, by contrast, treats a module's preservation state as a variable one that changes over evolutionary time and differs among modules and asks whether return remains possible.

A bottleneck core is a top-level regulatory node within a module that satisfies three criteria: loss of its function prevents coordinated transcriptional activation of the downstream module; no paralogous gene in the genome can completely or nearly completely substitute for its full regulatory function; and any compensatory pathway depends on the node itself. Non-compensability is the key evolutionary attribute, once the core is lost, the system has no alternative route to the module's functional output (e.g., Wu et al., 2026). A bottleneck core need not be a transcription factor; a key metabolic enzyme, a signal-integration hub, or a non-coding regulatory element can serve in particular lineages and modules (Wolfe, Tulloss & Pringle, 2012; Yona et al., 2013; Bayram & Braus, 2012). The mating-type loci work in a comparable way in a different regulatory context where single-locus switches whose loss collapses the

canonical sexual cycle, with the capacity to switch retained across lineages even as the locus architecture diverges (Heitman, 2015; Sun et al., 2025; Coelho et al., 2025); they are offered here as a structural analogy, not as instances satisfying the framework's criteria in full. A core's identity varies with lineage and developmental context, because nodes occupying the same functional position in the same ancestral network need not be strictly orthologous; lineage-specific expanded paralogs or convergently evolved molecules can serve instead. What is conserved is non-compensability. Two corollaries follow. Different trait transitions involve different numbers of bottleneck cores, and the decay rate of downstream networks after core loss also differs; these differences can give a preliminary basis for why different traits occupy different positions on the reversibility spectrum.

Wagner's character identity networks (ChINs) explain the origin and grounding of character identity (the historical continuity of a trait) through a shared regulatory core (Wagner, 2014). Bottleneck cores explain the loss of return accessibility after a character has been functionally abandoned. Both rest on the decisive role of top-level regulatory nodes, but they address distinct questions: the origin of novelty and identity, and the reversibility of character loss. The distinction between the origin of a novelty and its later exploitation has a long history in evolutionary thought (Erwin, 2015).

A parallel insight comes from the hierarchical GRN framework of Davidson and colleagues: deeply conserved, densely connected "kernels" are distinguished from peripheral subcircuits, and the evolutionary consequence of a change depends on its hierarchical position. Kernel changes produce irreversible shifts, peripheral changes permit reversible transitions (E. H. Davidson & Erwin, 2006; E. H. Davidson, 2010; Erwin & Davidson, 2009). In the present framework, conservation and connectivity guide the search for candidate bottleneck cores, but the decisive criterion is non-

compensability: whether any alternative route could restore coordinated module function once the core is lost. The two accounts diverge on two configurations. Where a conserved, well-connected node has an available alternative route, kernel logic predicts irreversible consequence; the present framework predicts reversibility, because the decisive property is non-compensability, not conservation or connectivity. Where a sparsely connected node has no alternative, the present framework predicts lock-in, whereas kernel logic does not generate this prediction.

Section III examines the pathway that generates evolutionary openness—the forward face of the decoupling identified in Section I—whereas Section IV examines how the loss of module integrity closes return paths, the reverse face of that same decoupling. The unit of analysis must first be fixed. The framework's unit is the module, not the trait. Traits relate to modules in three ways. A one-to-one relation occurs when a single module encodes the trait, as in a metabolic pathway or a single developmental switch. A many-to-one relation occurs when a composite structure assembles several modules. An ensemble relation occurs when an ecological strategy, such as ectomycorrhizal symbiosis or obligate parasitism, is realized through a suite of modules spanning signalling, colonization, and nutrient exchange. Predictions are formulated at the module grain, and composite or ensemble traits require explicit aggregation. The "reversibility of ectomycorrhizal symbiosis" summarizes the preservation states of the several modules whose joint activity constitutes the symbiosis; it is not a property of a single "symbiosis module." The expectations of Section V and the case formulations of Supplementary Box 2 follow this convention.

III. The diversity-generating pathway: how variation is supplied, retained, expanded, and assigned

The genetic–phenotypic decoupling identified in Section I runs through two layers, and this section is organized around both: genetic change can fail to reach phenotype, buffered by the canalizing systems of III(1); and genetic or phenotypic variation can fail to reach fitness consequences, sheltered by the plateau of III(2). Within this frame, the diversity-generating pathway sets the breadth of exploration a lineage can sustain and thus represents the forward face of decoupling. Module integrity, defined in the preceding section, sets the boundary of what the lineage can return to and thus represents the reverse face. Different processes maintain the two quantities. A lineage may hold high integrity in a module yet explore conservatively: strong stabilizing selection, ecological stability, or limited mutational input can constrain the pathway while the regulatory architecture remains intact. Conversely, a lineage may lose integrity in particular modules while the pathway continues through the rest. In gasteroid fungi, most modules remain active even as the module for open fruiting-body formation has eroded. The two quantities couple at one point only, and the coupling is causal in that the buffering and modular architecture that make variation cheap to accumulate are the same architecture whose loss lets erosion proceed unseen. The quasi-neutral plateau (Section III(2)) is both the medium through which drift explores and the entry channel through which bottleneck cores drift toward inactivation.

(1) Variation supply: modular development, co-option, and robustness

The developmental architecture of fungi is built from iterative hyphal units. A mycelium is a syncytial network of many semi-autonomous modules, such as individual hyphae or hyphal bundles. These modules are loosely coupled (Kües & Navarro-González, 2015; Nagy et al., 2017). Local variation therefore rarely triggers global collapse: an altered branching frequency, angle, or fusion pattern in one hypha is buffered by the rest of the network and by developmental plasticity. This construction

can structurally lower the threshold at which variation persists, while local changes seldom cascade to disrupt the functions of other modules.

Certain fungal groups commonly exhibit various types of fruiting-body malformation, including confluence, fasciation, proliferation, and inversion. (Kües & Navarro-González, 2015). Some may stem from mutations at a few specific loci or from environmental triggers, yet they often do not prevent effective spore dispersal. This tolerance of macroscopic deviation suggests that the underlying developmental program possesses a degree of robustness; under systemic perturbation or developmental reprogramming, modular construction may allow local morphology to deviate substantially without necessarily aborting the overall developmental process. The same property may also bear on the evolutionary origin of unusual forms such as the genus *Podoserpula*. Mechanistically, the visible phenotypes could arise via at least three broad routes: endogenous perturbation of key regulatory loci, whose effects may overwhelm molecular buffering networks; exogenous environmental inputs that alter developmental signal cascades; and pathogen-mediated interference, possibly acting through host responses such as RNAi that systematically perturb developmental modules. The routes differ in more than proximate cause. Exogenous and pathogen-associated perturbations need not pass through the buffering architecture that processes endogenous mutations, and so supply phenotypic variation through a partly separate channel. Because fungal development is modular and parameterized, such states can arise without genetic change and need not carry fitness costs that selection would register; they can persist as standing phenotypic variation. In some lineages they may later be brought under genetic control as mutations fixing within the quasi-neutral plateau of III(2) anchor them, a phenotype-first route whose fixation step is carried by drift, not selection. The structure parallels plasticity-led evolution (West-Eberhard,

2005; Ghalambor et al., 2007; Pfennig et al., 2010), but with anchoring by drift within the plateau rather than directional accommodation by selection; whether this route contributes materially to fungal macroevolution remains open, and its consequences for reversibility run through III(4) and Section IV.

The non-determinacy evidenced by such malformations may itself reflect the emergent character of fungal developmental organization. The hyphal tip can act as an integrative node for processing environmental and developmental information (Held et al., 2019), and models suggest that some aspects of fungal developmental organization arise from collective hyphal behaviour rather than from a centralized control hierarchy (Meskauskas, Fricker & Moore, 2004a; Meskauskas, McNulty & Moore, 2004b; Moore, 2005). The mycelium operates as a continuously reconfiguring network (Rayner, Ramsdale & Watkins, 1995; F. A. Davidson et al., 1996), with forms emerging at colony margins through processes that retain a degree of indeterminacy (Rayner et al., 1995; F. A. Davidson et al., 1996; Andrews, 1995). Vegetative fusion (Glass, Jacobson & Shiu, 2000; Rayner, 1991a) and the inherent flexibility of hyphal extension (Rayner, 1991b; Rayner et al., 1995; Voisey, 2010) further blur the boundaries between distinct developmental units. In such a system, modularity (the developmental and morphological organization of the fungal body into distinct units) may accordingly be less a fixed structural given than an emergent consequence of network dynamics, an attribute whose evolutionary significance may be greater in fungi than in lineages with more centralized and more determinate body plans (Rayner, 1991a; F. A. Davidson et al., 1996).

Development is also parameterized. Branching, elongation, fusion, and termination are fundamental operations, and much morphological diversity arises from adjusting their temporal and spatial parameters. This parametric architecture underlies co-option, the

redeployment of existing developmental modules into new functional contexts, and much of the diversification of complex fungal morphology can be read as this kind of re-parameterization (Kiss et al., 2019; Merényi et al., 2020; Krizsán et al., 2019; Nagy et al., 2023). Because co-option reuses modules rather than building them de novo, exploration of one module's parameter space need not disturb others, which lowers the cost of evolving complex traits. Within the constrained possibility space, convergence recurs; within it, the diversity of parameter combinations remains rich on evolutionarily accessible timescales.

Parameter recombination presupposes a stable identity core in each developmental module, specified by the top-level regulatory network rather than by current parameter values, in the sense of Wagner's character identity networks (Wagner, 2014). Co-option operates on a module's realization while the identity-conferring core remains intact. Realization here refers to a module's spatial and temporal parameters, and the same module identity can support multiple such realizations. Should the core be lost, the module ceases to be co-optable in this sense, leaving fragmented downstream residues subject to decay. The stability of the identity core during normal exploration is the architectural precondition of the flexibility that fungal development displays. Such a system is continuously reconfigured through growth, fusion, and local interaction. In certain respects, it can be seen as consonant with process-oriented accounts of biological organization, which view organisms less as fixed entities than as ongoing processes (Dupré & Nicholson, 2018).

Canalization buffers the variation this architecture supplies. Negative feedback in gene regulatory networks, redundant regulatory connections, and molecular chaperones such as Hsp90 and suppressor tRNA absorb perturbations at the levels of transcription, translation, and protein folding, so that some mutations never reach phenotype

(Rutherford & Lindquist, 1998; Cowen & Lindquist, 2005; Waddington, 1942; Leach et al., 2012). This buffering is a recognized structural basis of evolvability, the capacity to generate heritable phenotypic variation (Kirschner & Gerhart, 1998), developed further in the recent evolvability synthesis (Hansen & Wagner, 2023). In fungi, it can mask a standing pool of morphological potential, offering one reading of phenotypic noise and of the decoupling between morphological evolution and accumulated genetic differentiation (Bergman & Siegal, 2003). This is the first concrete layer of the decoupling. Buffering converts genetic change into silent variation and, by widening the plateau described in III(2), extends that silence to fitness itself. Buffering can also help maintain module integrity under weak or absent selection, but only while the core regulatory architecture remains intact: robustness absorbs small perturbations, not the decay that follows once a bottleneck core is inactivated (Section IV).

Modular construction, parameterized development, co-option, and canalization together supply a broad range of viable variation, which serves as the raw material on which the remaining processes operate, whatever the eventual fate of the lineage.

(2) Variation retention: the quasi-neutral fitness plateau

Variation survives when selection cannot discriminate its fitness effects. This section focuses on one situation of particular theoretical importance, the region in which fitness effects fall below the discrimination interval, though variation retention can be realized through multiple paths. The focal region is the quasi-neutral fitness plateau (Kimura, 1968; Ohta, 1973). A functional threshold structures the plateau. Where multiple genes jointly support a function and their joint output already meets the minimum threshold that selection requires in typical scenarios, fine-tuning of any single gene escapes strong directional constraint, and nearly neutral mutations accumulate as polymorphic sites or paralogous duplications. Selection still eliminates severe loss-of-function mutations

that drop output below the threshold; within the threshold, neutral exploration continues. A considerable fraction of mutations in protein-coding sequences falls in this near-zero interval, and slightly deleterious mutations fix slowly, so molecular rate limits can give a conservative account of how complex traits persist under drift-dominated conditions (Lynch, 2007). The threshold logic has empirical support in one well-studied system: wild-type protein activity often sits on a plateau safely removed from the fitness shoulder, and many mutations exert near-zero effects until expression is substantially reduced (Jiang et al., 2013).

In genotype space, the plateau corresponds to a neutral network and act as a connected set of fitness-equivalent genotypes through which populations drift without change in fitness (Wagner, 2008). The network's connectivity, breadth, and adjacency to other functional regions govern how efficiently drift explores and how readily new functions are reached. This drift does systematic architectural work under constructive neutral evolution. Complexity accumulates without positive selection, as mutations neutral in isolation fix and collectively establish new structural dependencies (Stoltzfus, 1999, 2012; Gray et al., 2010; Lukeš et al., 2011; Muñoz-Gómez et al., 2021). The quasi-neutral plateau is the fitness-space region in which this construction proceeds, and the closure it produces, entrenchment closure, differs from the closure this paper tracks. Nothing is lost: the components remain functional, and retention-based integrity, as operationalized in Supplementary Box 1, registers no loss. What closes is the path to simplification, namely that each dependency, dispensable when it arose, is now load-bearing and that dismantling any single one is deleterious; and the closure is no less real for being invisible to the retention measure. Entrenchment closes by construction; the erosion closure closes by destruction. The two nevertheless share a skeleton: in both, reversal demands a coordinated multi-step sequence that selection cannot favor

stepwise in the joint-probability sense of statistical inaccessibility (Section IV(1)).

The plateau's dual character bears directly on reversibility, and it is a duality of selective silence, not of construction: what selection cannot see, it neither purges nor preserves. A bottleneck core whose trait confers no current fitness advantage or disadvantage sits under no continuous directional selection; its inactivation can occur by drift, without immediate fitness penalty. Nearly neutral variation thus persists as material for future innovation, because selection fails to purge it; dispensable cores drift toward inactivation, because selection fails to preserve them. Exploration and erosion share a common starting point in the same drift-dominated region: the same silence that shelters future possibility also abandons the machinery of return. Construction and destruction can be serially coupled: a neutral addition that bypasses or duplicates an existing function silences selection on the original module, opening the first of the erosion routes of Section IV(1)).

Several factors modulate the plateau's effective width, and they differ across modules and lineages. Network architecture widens it in that epistatic interactions, redundant connections, and loose coupling among developmental modules buffer individual perturbations and confine the fitness cost of local variation to its own module. Loss of a downstream gene compensated by paralogs or alternative routes leaves the module's coordination intact, persists as polymorphism, and does not reduce module integrity. Such trait transitions appear frequently reversible in phylogenies. Pleiotropy narrows the plateau toward the core: bottleneck-core genes regulate numerous targets and bear substantially tighter constraints than downstream genes, so the plateau broadens at the periphery, where redundancy and weak pleiotropy prevail. Ecology sets the outer boundary. The plateau widens where carbon sources are diverse and mutually substitutable, a condition evident in lignocellulose-degrading saprotrophs (Camenzind

et al., 2024; Opulente et al., 2024) and, in varying degree, in mycorrhizal symbiosis, where host-supplied carbon weakens selection on the fungus's own acquisition machinery. It narrows in obligate parasitic lineages, where all carbon derives from the host. Mycelial network connectivity weakens local competition through resource redistribution (Simonin et al., 2012; Aguilar-Trigueros et al., 2022), an effect reduced in parasitic and lichenized lineages; in unicellular yeasts, which lack filamentous architecture, the tolerance space for morphological variation is smaller. Antagonistic pleiotropy under environmental fluctuation (Song et al., 2025; Mullis et al., 2022; Tellier, Villaréal & Giraud, 2007), environmentally induced module plasticity (Dan et al., 2026), and the activity of the buffering systems of Section III(1) further modulate the effective width.

Secondary-metabolite gene clusters illustrate the plateau's occupancy, though they do not test it. Functional redundancy, such as different clusters synthesizing the same metabolites, shows multiple genetic configurations producing equivalent outputs (Throckmorton et al., 2016; Forseth et al., 2013); pseudogenized and fragmented relics show configurations that selection does not efficiently eliminate (Ozturk et al., 2019; Balamurugan et al., 2024). Most clusters are transcriptionally silent under standard laboratory conditions, and their natural functions are difficult to ascertain (Chiang et al., 2011; Macheleidt et al., 2016); direct evidence of strong current selection is therefore lacking, though selection may operate under conditions not yet examined. A substantial fraction of biosynthetic gene clusters plausibly occupies the plateau: not efficiently purged, and not demonstrably maintained by strong selection.

Broad substrate tolerance points the same way. Many fungi degrade synthetic chemicals they have never encountered, such as dyes, chlorophenols, polychlorinated biphenyls, organophosphate pesticides, explosives, polycyclic aromatic hydrocarbons (Torres-

Farradá et al., 2024), through the broad specificity of laccases, peroxidases, and cytochromes P450 (Khan et al., 2023). Enzymes with no current direct fitness effect can therefore carry value at the level of the system, maintaining a toolkit from which responses to novel substrates are assembled. On this reading, the plateau is not a passive reservoir, it buffers functional capacity whose value is realized episodically, when a novel substrate or ecological opportunity appears. Extension from metabolism to developmental modules, which face stronger pleiotropic constraints, requires caution.

The plateau and canalization together locate the species-level pattern noted in the Introduction. Cryptic species complexes, defined by substantial genetic differentiation despite shallow morphological divergence, are the expected signature of quasi-neutral accumulation behind a buffered developmental program; environment-induced morphological divergence within a single genotype is the same buffering viewed from the other side. Genetic differentiation without morphological divergence, and morphological divergence without genetic differentiation, are thus two entries into one decoupling. When such diverged lineages coexist, the plateau finds a community-level counterpart where competitive differences among species are small, priority effects can override competitive exclusion, and community structure reflects drift as much as selection (Connell & Slatyer, 1977; Alford & Wilbur, 1985); closely related species with minimal niche differentiation can then persist in sympatry because stochastic colonization and priority effects jointly maintain them. Selective silence is thus not a single phenomenon but a family of phenomena. Molecular near-neutrality within genes, buffered accumulation within modules, and drift-dominated coexistence among species. Each has its own mechanism and evidence standard. These observations fit the account without distinguishing it.

Whether retained variation spreads, and whether the modules that generated it remain

intact, are separate questions, the first taken up in Section III(3), the second in Section IV.

(3) Variation expansion: the somatic evolutionary channel

For variation to reach the population level from a local region of the network, a channel of expansion is required, and many filamentous fungi plausibly possess one that operates in parallel with sexual reproduction. The mycelium is a syncytium in which nuclei migrate between connected hyphal segments: a mutation arising locally generates a population of nuclei through mitosis; these spread through hyphal tip growth and cytoplasmic flow; and through hyphal fusion, the mutant genotype can enter other regions and coexists with other genotypes within the same syncytium (Jinks, 1952; Roper et al., 2013; Strom & Bushley, 2016). The process involves no meiosis, no mating-type compatibility, and no discrete generations. Generation, spread, and local fixation of variation can be initiated, and possibly completed, within the somatic framework of a single network. The channel runs wherever the lineage ultimately falls on the reversibility spectrum, spreading whatever variation the modules supply, whether that variation later fuels exploration or accompanies erosion. Sex and clonality are not alternative states in fungi; many predominantly clonal lineages retain low-frequency sex (Nieuwenhuis & James, 2016).

Somatic architecture complicates the concept of effective population size. For fungi that form large mycelial networks and asexual propagules, the individual is ambiguous and N_e is correspondingly difficult to estimate (Taylor et al., 2015; Ali et al., 2016; Krueger-Hadfield et al., 2021; Ma et al., 2016; Booth, 2014); direct estimates in ectomycorrhizal fungi shift systematically with the degree of clonal reproduction and with population delimitation (Champion et al., 2026), and standard phylogenetic models do not accommodate variation in N_e across lineages (Borges et al., 2022).

The difficulty decomposes by organizational level. A nucleus-based framework for filamentous fungi distinguishes selection among nuclei within one cytoplasm, among nuclear subpopulations in different hyphal compartments, and among mycelia as wholes (Lakovic & Rillig, 2022; Sperschneider et al., 2023); somatic genetic variation in modular, clonal organisms nests within sexual reproduction, so classical frameworks require extension when several levels of selection operate simultaneously (Reusch, Baums & Werner, 2021). At the level of nuclei and compartments, the relevant dynamics are those of a somatic population expanding within a connected syncytium, a process standard N_e does not describe. At the level of genets and ramets, where the somatic channel interfaces with sexual reproduction and dispersal, N_e functions as a continuously modulating factor, and standard estimates capture this slice of a multi-level process rather than the whole.

The weight of N_e varies with life history. Where the somatic channel is well developed, N_e is one factor among several; where the channel is absent or drastically reduced (as in obligate parasites, endosymbionts, and unicellular yeasts), standard population-genetic dynamics regain much of their explanatory force. The expectation that smaller N_e accompanies inbreeding and asexuality, weakening the efficacy of selection (Whittle, Nygren & Johannesson, 2011), is borne out by elevated sequence evolution in endosymbiotic and parasitic fungi relative to free-living relatives (Woolfit & Bromham, 2003). Conversely, unexpectedly large N_e in asexual arbuscular mycorrhizal fungi suggests that population size can compensate for the disadvantages of clonality (VanKuren et al., 2013), though the asexuality of these fungi should not be overstated: in *Rhizophagus irregularis*, cytological and genomic evidence indicates a sexual origin of heterokaryosis (Ropars et al., 2016). Studies of partially clonal pathogens reach the same conclusion: clonal propagation and dispersal jointly shape genetic structure in

ways standard models do not capture (Barrès et al., 2008; Dutech et al., 2008).

When a hyphal segment carrying a mutation produces asexual spores, the mutant genome is packaged into clonal propagules with independent dispersal. Such propagules establish new colonies on ecological timescales and can, over phylogenetic timescales, form recognizable independent lineages. The somatic channel thereby releases the generation, retention, and lineage establishment of variation from the sexual cycle. A spatially extensive, long-lived network can explore different parameter configurations in different regions and export them at the periphery through spores (Anderson et al., 2018; Smith, Bruhn & Anderson, 1992); extensive mycelial networks capable of long-distance nutrient transport already existed in early terrestrial ecosystems (Vajda et al., 2023). The expected effect of this channel is an increase in the number of variants explored and exported within a single generation, and an extension of the period during which variants persist before loss or fixation, though the specific contribution in different lineages awaits empirical assessment, and the magnitude of the effect may prove modest where sexual reproduction predominates. Its efficiency should be higher in asexual clonal lineages, where new variants replicate and spread rapidly through spores; in groups more dependent on sexual reproduction, long-lived networks and frequent hyphal fusion still provide room for somatic evolution, at lower efficiency.

(4) Functional assignment: selective recycling

Traits maintained under drift-dominated dynamics can acquire adaptive value in a particular ecological or internal context and be refined under directional selection, a process termed selective recycling. The genotype-space picture gives this process a formal skeleton, though its application to fungal modules remains heuristic. A population drifts within a neutral network; at the network edge, one or a few mutational

steps can move the genotype into an adjacent functional network, a region of genotype space associated with a distinct phenotypic output and a different fitness regime. If the new function confers advantage, the genotype fixes and enters refinement under directional selection; if deleterious, it is purged, and the population retreats to the original network to continue exploration. Variation accumulated within a functionally equivalent zone can thus become the starting point of adaptive radiation, triggered by a few key mutations. In attractor terms, the system moves from a shallow basin into a new functional basin, altering its steady-state developmental output. Regulatory networks show the required temporal sequence: in the conserved regulator *Ndt80*, extensive drift of downstream target connections preceded the switch from meiosis regulation to biofilm formation (Nocedal, Mancera & Johnson, 2017), and in synthetic circuits, lost regulatory function has been regained through new mutations, not by restoring the original components (Kheir Gouda, Manhart & Balázsi, 2019). The distinction between the origin of a trait and its current maintenance is classical (Waddington, 1957).

Recycling proceeds through two non-exclusive pathways. In the exogenous pathway, ecological change confers adaptive value on a trait that drift had maintained at negligible fitness consequence, a morphological variant neutral in its ancestral setting acquires value when the lineage encounters a novel host or substrate. The variant need not have been genetically committed in advance: states generated by environment- or pathogen-induced reprogramming (Section III(1)) can supply the standing phenotypic front-end, with anchoring following within the plateau rather than preceding the ecological shift. Selection in this route acts late. In the endogenous pathway, accumulating systemic variation, combinatorial effects, or network rewiring give existing traits functional significance within a new internal context while the external

environment remains stable. Endogenous recycling draws on the combinatorial space of parameterized morphogenesis, where parameter adjustments fixed independently at two or more loci can combine within an individual into a configuration with novel function, and on the mycelial network itself, where metabolic or morphological variants scattered across different regions can be brought together by hyphal fusion and material transport, producing emergent functions that no single region could achieve. Rich intermediate situations exist between the two poles. Horizontal gene transfer adds a further route at the molecular level: complete modules acquired in bulk from distant lineages compensate module integrity from outside rather than through recycling (Reynolds et al., 2017; Chrismas et al., 2025; Section IV, Supplementary Box 2).

One observable pattern fits the recycling reading: when a novel trait combines with a particular ecological niche, net diversification rate increases (Sánchez-García et al., 2020; Wilson, Binder & Hibbett, 2011). Diversification rate is not a proxy for module integrity. It can rise in high-integrity lineages, where broad exploration supplies abundant material for recycling, and in low-integrity lineages, where an already constrained trait enters a new niche. Selective recycling mediates the relationship between the two, and in empirical tests they are treated as independent variables.

The four processes together supply, retain, expand, and assign variation, setting the breadth of exploration a lineage can sustain. They do not maintain the modules through which they run. Integrity is maintained by purifying selection and developmental robustness, each effective under its own conditions, and by contingent factors such as intermittent recycling, pleiotropic coupling, and low-frequency recombination. The pathway is not one of these contingent factors. Where the pathway is active, reversibility among shallow attractor basins follows from exploration under drift. Whether a lost trait can return is set by the preservation state of the module that encoded

it is the question that Section IV takes up.

IV. Where exploration leads: the emergence of directional constraint

Section III described the machinery that sets the breadth of exploration; this section describes what happens when module integrity declines, and how that decline closes return paths. Where the developmental-constraints tradition asked why some variants never appear (Maynard Smith et al., 1985), the question here is why some variants, once they have appeared, cannot reappear. The decline is stealthy in a specific way, and same buffering that makes the forward direction cheap hides the erosion of coordination until a bottleneck core is lost. This is the asymmetry announced in Section I, now given a mechanism.

The properties that make exploration possible carry an inherent fragility. Shallow attractor basins permit transitions among states with small genetic changes, including entry into trajectories that end in directional constraint. A lineage can move into a dependent or truncated state through steps modest enough to be accommodated within the quasi-neutral plateau, not because the steps are adaptive but because they are not costly enough to be purged. Constraint is not a separate mode of evolution opposing exploration but one outcome of exploration itself. The outcome of paths that involve the loss of bottleneck cores and the degradation of their downstream connections. This fragility is a consequence of the modular architecture, not a failure of it. Same shallow-basin structure that permits drift-driven exploration permits drift-driven entry into trajectories from which return is statistically constrained.

The erosion is module-specific. A lineage may be deeply constrained in one trait while its other modules continue to explore; gasteroid fungi have lost the module for open

fruiting-body formation while other developmental and metabolic modules remain intact. The conditions under which particular modules cross the critical zone, and the consequences for the traits they encode, occupy the remainder of this section.

(1) Pathways of reduced integrity

Three supports maintain module integrity. Purifying selection is most effective while a module remains functionally recruited and its output approaches the lower bound of the viability threshold. Developmental robustness buffers perturbations, not functional loss itself, and only while the module's core regulatory architecture is intact. Contingent factors contribute in ways a selection-and-robustness formulation does not capture: intermittent selective recycling, pleiotropic coupling with other modules, low-frequency recombination. A module that is presently neutral need not have been neutral throughout its history. A module may cease to be recruited altogether when it loses its regulatory connectivity, network topology, and coordinated expression, whether through bottleneck-core inactivation or through other routes such as relaxed selection, robustness can no longer act directly, and when the contingent supports also fail, mutation gradually erodes the module and its interfaces with other modules: gene content declines, topology disintegrates, integrity decreases. Two routes enter this sequence.

In the first, regulatory change makes a module dispensable to the current life cycle. If pleiotropy or other factors cannot constrain it, the bottleneck core and some of its downstream genes enter a strictly neutral zone. In large populations the stable fixation of nearly neutral mutations is slow, and specific loss-of-function mutations arise rarely; for a considerable period after relaxation, such alleles persist as low-frequency polymorphisms while functionally intact alleles remain, leaving room for early evolutionary reversal. The buffering of Section III(1) absorbs mutations accumulating

in the module and its targets while the core stays functional; once the core is inactivated, the epistatic buffering organized through it disintegrates (Section IV(2)).

In the second, ecological transition abandons the module wholesale, such as the host supply of particular metabolites, or a new strategy that bypasses the original functional requirement, as documented in obligate biotrophs whose genomes have lost entire biosynthetic pathways (Cissé, Pagni & Hauser, 2014; Spanu et al., 2010). All genes of the module escape functional constraint and, absent pleiotropy, degrade at similar rates. Initial reversibility in this route depends on the degradation mutations already accumulated across the module; retention of the bottleneck core is one aspect among several.

The two routes converge on the same endpoint. As loss-of-function alleles of the bottleneck core become fixed, as initial cause or subsequent result, downstream targets lose their center of coordinated expression and pass into functional inactivity. As inactivating mutations accumulate nearly neutrally, regulatory connections break, coding sequences become pseudogenes, and entire genes are lost. These changes disperse across many loci with individually small effects, and selection does not easily purge them. Once degradation passes that the point at which the joint probability of independent mutational events required to restore complete module function has fallen to an extremely low level. Trait reversal may become statistically inaccessible, and directional constraint is thereby established. Hereafter, "statistical inaccessibility" denotes this joint-probability sense. The constraint is trait-specific: it closes the return path of the function this module encoded, while other modules of the same lineage continue to supply exploration through the pathway of Section III.

Specialization proceeds by addition or by subtraction, and the two label directions of change at the module grain rather than positions on a single axis. Additive

specialization, such as gene-family expansion, regulatory adjustment, horizontally acquired capacities extends the repertoire while ancestral free-living modules remain largely intact; and it can proceed passively under near-neutral conditions, consistent with the zero-force expectation that unconstrained systems drift toward greater diversity (McShea & Brandon, 2010) and with the constructive-neutral account of complexity arising without positive selection (Stoltzfus, 1999, 2012).

Subtractive specialization erodes module integrity directly; additive specialization leaves integrity intact, though its additions are not thereby freely reversible. Entrenchment (Section III(2)) can wire them into load-bearing dependencies, closing simplification without erosion. Many actual transformations combine both, for example co-option and re-parameterization expand one component while contracting or rearranging another. The modes differ in their consequences for reversibility (additive specialization need not move a lineage toward the critical zone, whereas subtractive specialization does), and the framework's expectations apply with correspondingly different force (Supplementary Box 2, case 9).

Subtraction itself is heterogeneous. Gene loss under relaxed selection proceeds through near-neutral pseudogenization, through adaptive deletion favoured for its resource savings, or through co-elimination of functionally linked genes as a unit (Albalat & Cañestro, 2016); the mechanisms differ in tempo and molecular detail but converge on the dismantling of module integrity, with adaptive and co-eliminative loss reaching the endpoint faster than drift alone. Amplifiers of a shared trajectory rather than competing explanations. The tempo should therefore vary with the kind of module. Metabolic pathways made redundant by host provisioning invite adaptive deletion and erode rapidly; neutrally lost modules persist in partially degraded form over extended timescales; functionally integrated modules are neither adaptively deletable nor

neutrally degradable, and purifying selection and robustness hold them in place. This differential vulnerability grounds the differing evolutionary fates of traits in the organization of their underlying modules, without invoking a single universal rate of decay. The residuals of erosion, whether produced adaptively or neutrally, can in principle supply material for selective recycling, just as variation accumulated within the plateau does (Section III(4)). Such material may arise through gene loss that releases previously suppressed alternative pathways, through pseudogenized sequences that supply cis-regulatory material for neighbouring loci, and through simplification that dissolves pleiotropic or epistatic constraints which had limited variation (Albalat & Cañestro, 2016).

(2) Architecture, not gene content

Loss of the bottleneck core is accompanied by relaxation of pleiotropic constraints. Under normal conditions, bottleneck-core genes bear strict structural-functional constraints; their minor biophysical perturbations are amplified by the downstream network, and the system requires epistatic buffering to hold its output stable. Once the core is inactivated, this constraint can lift: purifying selection on downstream genes weakens and degradation accelerates. The epistatic buffering previously organized through the core loses its coordination center and may gradually disintegrate; the shrinkage of transcription-factor repertoires in obligate host-associated fungi may be read as a systemic manifestation of such regulatory-module erosion (Möller & Stukenbrock, 2017). The collapse of the pleiotropic constraint layer is itself a dimension of module integrity reduction.

Losses that do not involve top-level nodes behave differently. Where a downstream gene's function is compensated by paralogs within the module or by redundant regulatory connections (Wagner, 2005), its loss is nearly neutral within the functional

equivalence zone. One gene's loss is masked by another's upregulation, and phenotypic output is unchanged. Such losses persist as polymorphisms, trigger no systematic relaxation of constraint, and leave module integrity intact; even uncompensated local defects stay confined within the module by developmental robustness, and global collapse is unlikely. What matters for reversibility is the loss of non-compensable coordination. The decisive change is architectural, not numerical.

The dimorphic fission yeasts illustrate the architectural reading, within the limits of the evidence. *Schizosaccharomyces japonicus* retains hyphal growth, whereas *S. pombe* has lost it; one comparative analysis suggests that the difference lies not in the presence or absence of hyphal-development genes but in genome rearrangements that may have altered the regulatory architecture connecting those genes to the hyphal program (Papp et al., 2021). This interpretation rests on a single study, and the causal link between the observed rearrangements and the loss of hyphal growth has not been independently confirmed. Plausible alternatives remain, including differential regulation of downstream effectors and changes in signal transduction. If supported across additional lineages, the case would show gene content remaining largely in place while module integrity declines: topology, rather than gene count, as the decisive difference. The complementary configuration is documented in dimorphic Mucorales, where yeast and hyphal programs are supported by differentially functionalized paralogous families within a single genome (Tahiri et al., 2026), which retained functional coverage supplied by paralogs rather than orthologs, a distinction the measurement design of Supplementary Box 1 accommodates. This observation likewise awaits independent replication.

In the attractor description, a formal account of multistable gene regulatory dynamics (Huang et al., 2005), used here as heuristic for the accessibility of morphological states

— higher module integrity corresponds to a broad, shallow landscape: multiple attractor basins coexist, barriers between them are low, and the system moves relatively freely among morphological states. Loss of the bottleneck core blocks the paths to certain basins, and the landscape simplifies into a smaller number of deep, narrow basins restricting the system to one or a few accessible states. In neutral-network terms, connectivity declines, the network fragments or shrinks, and the system is confined within a reduced equivalence region (Wagner, 2008), the path back to the original function becomes statistically inaccessible, and the space for acquiring new functions may compress. These architectural readings await comparative and experimental grounding; the image of evolution as movement on a surface of selective value is classical (Wright, 1932, 1988).

Pleiotropy can contribute to the retention of individual genes, but its effectiveness in delaying module degradation is architecture-dependent. Downstream effectors retained through pleiotropy do not by themselves preserve the module's functional architecture: once coordinated expression disintegrates, retained downstream genes offer little toward restoration. Pleiotropy acting on the bottleneck core itself can delay degradation, provided its other functions remain under selection strong enough to purge loss-of-function mutations; the delay is partial, confined to the pleiotropic sites, and unlikely to postpone the module's decline substantially when those sites are few or weakly constrained. The maintenance value of pleiotropy concentrates at the core, diminishes toward the periphery, and is conditional even there.

Non-compensability, finally, is not a fixed attribute of a node but an evolvable one. When a module contains several functionally redundant regulatory nodes, their independent losses are individually near-neutral until the last is reached; the final node then acquires the evolutionary role of a bottleneck core even if it did not initially occupy

one. Entrenchment supplies the additive route to the same outcome: a node whose function later additions come to require is, for the current architecture, a bottleneck core, though nothing was lost (Section III(2)). Gene duplication followed by functional divergence could create compensatory alternatives and reduce a core's non-compensability; loss of a previously redundant paralog could render a compensable node bottleneck-like; regulatory rewiring, altered pleiotropic coupling, or co-option into a different developmental context could shift a node's position on the compensability spectrum. Non-compensability is thus a historically contingent property of regulatory architecture, and the framework's reversibility predictions apply to the current state of a module: what matters for return is the distribution of non-compensability at the time of trait loss, itself reshaped by prior duplication, loss, and functional turnover.

(3) The critical zone

The effect of integrity loss on reversal is expected to be nonlinear. As long as compensatory pathways and downstream targets remain intact, they can still support reactivation of the module. A possible threshold is the functional loss of the last non-redundant bottleneck core, or degradation of its downstream connections to the point where restoration would require multiple mutations and is therefore unlikely. Beyond that threshold, reversal probability should fall at an accelerating rate as integrity declines into a critical zone. Beyond the zone, reversal is strongly restricted on macroevolutionary timescales and appears in phylogenies as directional constraint, trait-specific as always: restoration would require rebuilding a coordination architecture that no longer exists. Reversibility is a function of the module's preservation state. Where integrity is high, a lost trait can still return; where integrity is low, it is statistically out of reach.

The critical zone is a region of accelerated decline, not a threshold that a single measurement could locate with precision. Within it, the restoration problem changes character, from the repair of isolated defects to the reconstruction of a coordination structure that no longer exists. A smooth but steep nonlinearity in the underlying reversal probability can appear in the phylogenetic record, at macroevolutionary granularity, as a discrete transition between reversible and constrained regimes. The framework specifies the qualitative form of the relationship, like nonlinear, trait-specific, conditioned by architecture, but not the location of the zone for any given module; its parameters are unknown.

The zone's location also need not be uniform across the kingdom, because the depth of achievable erosion depends on how much architecture there was to erode. Architectural depth, defined as the structural complexity of the ancestral module in terms of its number of hierarchical regulatory layers, the integration among its components, and the size of the target sets commanded by its cores, is a property of the ancestral reference state. Module integrity measures how much of that architecture a given lineage has preserved. Erosion should scale with ancestral depth. Where developmental systems are simple, with few modules and cores commanding modest target sets, specialization can impose only bounded erosion, and the scale of what must be restored remains limited; where development is deeply modular and cores command large target sets, the same relaxation drives erosion more extensively, and the joint probability of restoration falls accordingly. The contrast between early-diverging and higher fungi may offer a kingdom-scale illustration (Supplementary Box 2, case 9); the existence of such a pattern has not been demonstrated.

The temporal structure of the transition is visible in the gasteroid case. In *Lentinus tigrinus*, agaricoid and secotioid fruiting bodies are produced by the same genetic

background, with the morphological difference attributed to variation at a single locus (Wu et al., 2018; Supplementary Box 2, case 2), an existence proof that a dramatic morphological transition can be developmentally feasible with minimal genetic change. Populations in which gasteroid and agaricoid morphologies coexist point the same way: there is a temporal gap between allelic polymorphism at the individual level and evolutionary locking at the phylogenetic level. Reversal remains possible in the initial stage; as bottleneck cores are lost and downstream regulatory connections degrade, the threshold for reversal continues to rise. Whether, and how far, this mechanism applies to other gasteroid lineages would benefit from explicit testing.

(4) Why transitions differ

Trait transitions differ in the strength of directional constraint, and the regulatory hierarchy of the genes involved supplies the reason. Transitions involving peripheral nodes, or non-bottleneck regulatory genes with paralogous compensation, switch between shallow attractor basins and appear frequently reversible in phylogenies. Transitions involving loss of bottleneck cores restrict the system to a deep, narrow basin; the path back requires not only multiple independent mutations at the sequence level but the reconstruction, at the network level, of a coordination structure that has disintegrated. The number of genes involved bears no simple deterministic relationship to reversibility, and its association with the morphological complexity of the transition is even weaker. What matters is whether those genes constitute bottleneck cores with scarce compensatory pathways.

Between these poles lies a third class: lateral movement among related specialized states. Parasitic lifestyles are narrow relative to free-living strategies, but they contain multiple connected basins, such as entomopathogeny, mycoparasitism, phytopathogeny, and transitions among them recur. Clavicipitaceae have shifted among plant, insect, and

fungal hosts (Spatafora et al., 2007); lineages such as *Tyrannicordyceps* have crossed kingdom boundaries in successive steps (Kepler et al., 2012); truffle parasites derive from insect pathogens that shared their underground microhabitat (Nikoh & Fukatsu, 2000); many fungi infect both plants and humans (Gauthier & Keller, 2013), and inter-phylum jumps continue to be described among newly recognized taxa (Chen et al., 2024). Genomic analyses suggest that such jumps proceed through comparatively modular changes, including secretome remodeling and expression shifts rather than wholesale genomic reorganization (Quandt, Patterson & Spatafora, 2018), and that pathogenicity toward novel hosts builds on traits shaped by prior hosts (van Baarlen et al., 2007). These transitions mainly modify peripheral modules, such as effectors, receptors, and secretome components, while the core architecture of the parasitic toolkit remains intact. The same Dikarya lineages have not, to current knowledge, re-crossed the barrier to free-living existence; doing so would require rebuilding the eroded free-living modules. Lateral mobility within a regime and a closed exit from it are both expected features of the same architecture. Lateral mobility extends beyond parasitic modes: the rice endophyte *Falciphora oryzae* (syn. *Harpophora oryzae*) appears to have evolved from a pathogenic ancestor toward mutualistic endophytism (Xu et al., 2014), a transition that remains within the host-associated sector.

The breadth of exploration and the possibility of return are set by different quantities: the pathway sets the first, module integrity the second. Openness and constraint are not rival explanations of fungal macroevolution, but two regimes of the same evolutionary process, the process that determines reversibility.

V. Expectations and tests

We acknowledge that, at present, direct causal evidence is not yet sufficient to support the framework's core claims in full. Therefore, we order the eight expectations below by their evidential role. We begin with the association test and its design (E1, E2), then turn to the discriminating sequence test (E3), followed by corroborating molecular and comparative patterns (E4-E6), and finally prospective regularities (E7, E8). We present these expectations as separate points so that each can be assessed on its own terms and linked to the evidence that would confirm or challenge it. For each expectation we provide a grade, a status, and an assessment of what conflicting evidence would cost the framework. Grades indicate the stakes involved and are not a measure of evidential weight. E4, for example, is graded peripheral because its failure would require refinement, yet it is the only expectation with direct partial existing support. Drawing these boundaries in advance strengthens testability without shielding the framework from evidence. The graded approach follows recent calls for mechanistic tests of Dollo's law (Elmer & Clobert, 2024) and for measuring irreversibility along a continuum (Farina, Faurby & Silvestro, 2023), and extends them one step further: not only whether a given transition is irreversible, but where on the reversibility spectrum a lineage falls, and why. (Table 1 summarizes the expectations, their grades, and their current status.)

(1) The core test and its design

The core test asks whether independently measured module integrity associates with reversal probability across lineages. Its obstacle is a confound with a specific structure. Ecological release at the sorting stage simultaneously degrades the module through relaxed selection and removes the pressure for reversal, so that low integrity and absent reversal share one cause, namely downstream degradation that mimics the signal of a regeneration-stage gate. The association acquires evidential force only within designs

that separate the stages. In advance, we specify several features: (i) stratification by time since trait loss, within which measured integrity varies; (ii) sister-lineage contrasts holding ecology and biogeography constant; (iii) explicit accounting, not silent exclusion, of reversals accompanied by horizontal acquisition of module components, their frequency reported as itself an observable (Section VI); and (iv) direction-of-degradation checks (Expectation 3) confirming that the integrity measure reflects coordination loss rather than elapsed relaxed selection. The common-cause account predicts that reversal probability tracks loss-age and ecology. The framework predicts that, at matched loss-age and matched ecology, it tracks measured module integrity.

(a) Expectation 1 (core; testable in principle). Within a homologous framework, lineages with higher measured module integrity are predicted to show, on average, a higher probability of reversal from derived states than lineages with lower integrity. We designed the measurement to be independent of the outcome. We infer integrity from the module's own state, including its gene content, regulatory topology, and expression responsiveness, and we observe reversal as a separate phylogenetic event. The association is therefore not circular, because the same evidence does not both define and validate the construct. The independence is imperfect in practice. Reversal events and integrity measurements may share upstream causes (e.g., ecological stability, population history, and genomic architecture); moreover, some locked lineages may be closed by construction rather than through erosion (Section III(2)). The stratification designs above address the first complication; the signature contrast of Expectation 5, the second. We should specify the measurement-error model and the confounding model before examining the reversal data. This minimal pre-registration commitment preserves the independence claim in practice, not only in principle. Should the association prove absent once measurement error and shared-cause confounding are

explicitly incorporated, the core inference of the framework fails.

(b) Expectation 2 (core; conceptual). Reversal probability is expected to decline nonlinearly with integrity, most steeply within the critical zone of Section IV(3). The expectation is qualitative; where threshold models outperform linear alternatives is part of what the measurement programme would estimate (Supplementary Box 3).

(2) The discriminating test

Expectation 3 is the only prediction that discriminates directly between the two stage-specific hypotheses distinguished in the Introduction. Under the regeneration-stage gate, the earliest degradation should be ordered by regulatory position; under downstream release at the sorting stage, it should be ordered by gene-level susceptibility.

(a) Expectation 3 (core corollary; conceptual, with specified operational boundaries). In lineages that have lost a trait, the earliest relaxation of selection should be detectable at the bottleneck core and its regulatory connections before, or at least not after, structural degradation of downstream targets. The sequence claim is a corollary of the framework's logic, not an established empirical regularity; its function is to separate the unified-core account from independent degradation, a separation that coordinated degradation alone cannot deliver, since both accounts predict that gene content, network topology, and expression responsiveness deteriorate once selection relaxes. A null model gives the sequence claim concrete form. Rank genes within a degraded module by order of relaxation (e.g., dN/dS trajectories, pseudogenization timing, or loss polymorphism across a dated phylogeny) and fit this rank as an ordinal response with gene-level susceptibility covariates such as mutation target size, expression level, codon usage, pleiotropic coupling, paralog count. The unified-core account predicts that top-level nodes relax earlier than the covariates alone would

predict. These nodes are identified by criteria independent of the degradation data, such as conserved cis-regulatory motifs, synteny, and regulatory hierarchies from model systems. Non-random but position-independent ordering supports independent degradation with biased susceptibility; position-dependent ordering supports the unified-core account. Deviations attributable to measurement error, pleiotropic interference from functions external to the module, or lineage-specific alternative pathways remain compatible with the core logic; systematic reversal of the predicted order does not. The thresholds separating these outcomes require the calibrating data themselves; the directional commitment does not. We still need to construct an empirically grounded null distribution, whether from simulation or from modules whose degradation demonstrably involved no core. Until then, the sequence claim constrains research design rather than providing a decisive test.

(3) Corroborating and prospective patterns

The remaining expectations draw on evidence that exists but does not yet bear on the core claims directly: comparative phylogenetic patterns, molecular signatures, and genomic surveys.

(a) Expectation 4 (peripheral; testable in part). The functional roles of horizontally acquired genes should differ between integrity regimes. Where a lineage has lost the capacity of a particular module, a core metabolic pathway relinquished under obligate association, acquired genes should disproportionately compensate the functions of that eroded module; where integrity remains high, acquired genes should more often extend peripheral metabolic capacities. The expectation concerns functional roles, not absolute transfer counts: transfer counts respond to many factors of which integrity is one, transferred genes are frequently lost again (Richards et al., 2011), and transfer inferences are sensitive to methods and taxon sampling (Szöllősi et al., 2015). Available

evidence is consistent with the functional-category reading. In chytrids, acquired genes accompany lifestyle transitions and expand metabolic capacity rather than compensating eroded cores (Chrismas et al., 2025); microsporidia combine massive gene loss with recurring acquisitions (Corradi, 2015); the distribution of a secondary-metabolite cluster across hundreds of genomes reflects gene loss and transfer acting together (Reynolds et al., 2017); and a survey of 829 representative fungal genomes identified more than 20,000 prokaryote-derived genes, with parasitic fungi harbouring the highest numbers, gasteroid (closed-fruitbody) lineages the highest among morphological states, and wood saprotrophs enriched in transporters, signalling components, and protein-folding factors which are auxiliary categories rather than core free-living metabolism (Liu et al., 2025). None of these patterns tests the expectation directly; the test requires classifying acquisitions by the specific module they compensate and comparing across integrity regimes. The same material raises a further possibility that horizontal transfer facilitates irreversible transitions away from free-living lifestyles and no reversal from parasitism to a free-living state having been documented among the Dikarya, which remains untested.

(b) Expectation 5 (core; testable in principle). Lineages identified as locked by phylogenetic analysis are expected to show coordinated degradation signals at their bottleneck cores, such as elevated dN/dS ratios, enrichment of pseudogenization, and disintegration of co-expression networks, whereas flexible lineages are not expected to show these signals. Locked lineages lacking these signatures, where measurement is adequate, would point to entrenchment closure rather than erosion. The two closures should in principle leave opposite signatures: relaxation at the abandoned module for erosion, tightened constraint, dependence below the ancestral level, at load-bearing components for entrenchment (Section III(2)). One existence case is available: the

repeated, convergent loss of *abaA* in lineages that have abandoned phialide formation (Mead et al., 2020). The mycorrhizal gradient of Supplementary Box 2 (case 4) offers a complementary contrast at the genomic level. Systematic contrasts remain untested.

(c) Expectation 6 (core-adjacent; testable in principle). The probability of reversal from specialized to free-living states is expected to vary with the architectural depth of the modules involved (Section IV(3)). Specifically, reversal may be more frequent in lineages with shallow, weakly integrated architectures than in those with deeply nested, interdependent ones, though such an association would be probabilistic and context-dependent. Architectural depth is estimated per clade against that clade's own ancestral baseline; the kingdom-scale expectation is a qualitative pattern over clade-level results, not a comparison of absolute integrity values across baselines (Section II). Phylogenomic evidence of multiple independent transitions between parasitism and saprotrophy across the chytrid phylum (Thomé et al., 2024) is consistent with the shallow-architecture end of the gradient; a single observation of this kind does not by itself place strong constraints on the broader gradient. Until architectural depth is operationalized, apparent patterns are difficult to distinguish from sampling biases, timescale effects, or methodological differences in ancestral-state reconstruction.

(d) Expectation 7 (peripheral; prospective). The somatic channel may prove more consequential in lineages with extensive clonal reproduction and long-lived mycelial networks, which may show greater morphological disparity at comparable integrity levels. We make no strong commitment to a specific quantitative form in the framework and have yet to assess its contribution in different lineages.

(e) Expectation 8 (peripheral; conceptual). Transitions between parasitic modes may involve fewer and more modular genomic changes than transitions between parasitism and saprotrophy; lineages with histories of repeated host jumping may likewise share

intrinsic genomic signatures that strict specialists lack, such as genome-wide codon optimization correlated with host range (Badet et al., 2017). Convergent molecular adaptations between independently evolved phytoparasites (Kemen & Jones, 2012) and trans-kingdom convergence among endoparasites (Thorogood et al., 2021) are consistent with the premise; the transition-count prediction itself has not been quantified.

(4) Testing approaches

For the expectations that are testable in principle, some complementary approaches like phylogenetic logistic regression, threshold models, and covariate extensions of state-dependent diversification models provide a progressive test from event probability to transition rate; their designs, assumptions, and interpretive constraints appear in Supplementary Box 3. Whatever comparative approach we adopt, macro-patterns gain force when independent molecular evidence corroborates them. As Expectation 5 predicts, a lineage with reduced reversal probability should show coordinated degradation at its bottleneck cores. Phylogenetic analysis can then identify locked and flexible lineages as contrastive designs for functional validation. Together, macro-pattern and micro-mechanism form a reciprocal verification loop.

VI. Scope, boundaries, and the road ahead

Our framework operates on macroevolutionary timescales. It does not extend to the phenotypic plasticity of ecological time, such as the reversible morphological switches an individual executes within its lifetime, governed by epigenetic and transcriptomic mechanisms (Dan et al., 2026) rather than by the erosion of module integrity. Plasticity nonetheless enters the framework indirectly, as the phenotypic front-end of the non-

genetic supply channel of Section III(1): within-lifetime switches lie outside what integrity measures, but the states they generate can be anchored genetically under drift and acquire macroevolutionary standing. Our framework's variable remains integrity; plasticity is an input to the pathway, not a second quantity. The framework's principal domain is the Dikarya, and within that subkingdom chiefly the filamentous lineages; among unicellular groups, the relevant dynamics play out in metabolic and life-history evolution rather than in morphology. The early-diverging fungi mark this boundary constructively. In chytrids, architectural depth appears to constrain erosion in the nutritional domain, where bidirectional parasitism-saprotrophy transitions have been documented. (Thomé et al., 2024); what differs is the weight of the modulating factors, the somatic channel (Section III(3)) and the module-coupling conditions of Section III(2) being less developed in simple, often unicellular architectures. The chytrid gradient is the framework's natural outgroup test (Expectation 6), not territory beyond its reach. Within the Dikarya, the framework claims no universal exclusivity: it is a previously neglected, testable logic that may account for at least some lineages, and heterogeneity among clades is acknowledged rather than denied, including closure by entrenchment, which leaves module integrity intact and therefore lies outside what the variable measures (Section III(2)).

The pathways of integrity reduction are diverse and may be superimposed in any given case. Rare macromutational events, especially horizontal gene transfer, can bypass stepwise restoration by importing functional modules wholesale, and thus mark the boundary of the framework's ordinary logic. HGT-mediated restoration, where it occurs, falls under Case C of Supplementary Box 1: it restores the phenotype through a non-homologous route without restoring ancestral module integrity in the framework's sense. The exception is bounded rather than open-ended. Its frequency is an observable: the

rate at which phenotypic reversal is accompanied by horizontal acquisition of module components can be estimated in any clade with genomic sampling. The framework expects such events to remain rare relative to peripheral and compensating acquisitions (Expectation 4); a clade in which trait reversal routinely travels by horizontal acquisition would contract the framework's domain.

Module integrity is measured independently of the presence or absence of reversal. Design independence of this kind is a necessary condition for falsifiability, not a sufficient one: causal identification additionally requires the stratification and contrast designs specified in Section V. The framework claims the former outright and specifies the latter as part of its test protocol. It does not, separately, require distinguishing drift from weak selection at macroevolutionary scales. Structural features of fungal biology may systematically expand the domain in which selection signals are weak relative to drift noise; within that expanded domain, drift and weak selection jointly shape the observed pattern (Wang et al., 2023). The account is integrative rather than competitive: drift, weak selection, adaptation, and horizontal gene transfer enter a single causal narrative as components, a stance shared by the broader movement in contemporary evolutionary theory (Pigliucci & Müller, 2010; Laland et al., 2015).

"Statistical inaccessibility" is used throughout in its qualitative, joint-probability sense (Section IV(1)). A full analysis of what kind of impossibility it is, that is, of its modal structure, its thresholds, and their epistemic status, is deliberately beyond this paper's scope.

The framework's relation to evolvability research completes its theoretical coordinates. Module integrity concerns the preservation of a module's functional architecture; variational modularity concerns the structure of pleiotropic constraints; canalization concerns insensitivity to perturbation. These factors influence evolvability, understood

here as the capacity to generate heritable phenotypic variation (Kirschner & Gerhart, 1998), through partly distinct mechanisms, and evolvability has been proposed as a unifying concept (Hansen & Wagner, 2023). Module integrity contributes to that programme at a level that systemic statistical indicators do not reach: the evolvability of a particular module. An architecturally intact module remains open to re-parameterization, co-option, and selective recycling; a trait it encodes, if lost, may remain recoverable; its mutational neighborhood stays explorable. Integrity is a dimension of evolvability at the level of the individual module, not a proxy for the systemic capacity of the lineage as a whole. The two levels can diverge: a lineage may hold one module at low integrity while others remain intact, so that systemic capacity persists even where a particular return path is closed. Constraint without global closure is the framework's specific addition to the evolvability discussion, and it stands in a lineage of constraint-sensitive theorizing that runs from the spandrels critique of adaptationist explanation (Gould & Lewontin, 1979), through the developmental-constraints programme (Maynard Smith et al., 1985), to the evolvability literature just invoked.

The central variable remains unmeasured and the core association untested, and the next steps have a natural order. An initial priority is a proof-of-concept estimate of module integrity for one module across one gradient covered by existing data. We could focus, for example, on the plant-cell-wall-degrading modules of the mycorrhizal gradient, the *abaA* module across phialide-loss lineages, or the *Schizosaccharomyces* pair, computed from published genomes at whatever precision they allow. Such an estimate would not test the core claim; it would demonstrate that the construct is estimable, and locate the obstacles to estimating it well. The full agenda follows from that foundation: the measurement design of Supplementary Box 1 in a tractable homologous framework,

the bottleneck-core identification protocol, the comparative approaches of Supplementary Box 3, and the molecular corroboration of Expectation 5. One further extension follows naturally. A signed topology dimension that counts gained dependencies whose removal collapses module output would allow us to measure entrenchment load, or closure by construction, as a second axis alongside integrity. No part of this agenda has yet been executed with module integrity as the focal variable.

The account rests on two quantities with distinct determinants. The diversity-generating pathway sets the breadth of exploration a lineage can sustain. Module integrity, maintained conditionally by purifying selection and developmental robustness and eroded by the loss of bottleneck cores, sets the boundary of what it can return to. The two couple at the quasi-neutral plateau and nowhere else. In the terms of Section I, the pathway supplies the forward face of the genetic–phenotypic decoupling and integrity governs its reverse face; this is the asymmetry with which the Introduction opened, namely one architecture read in opposite time directions. Openness and constraint are two facets of a single process, namely the process that determines reversibility. Constraint is also module-specific, a lineage may be locked in one trait while its other modules continue to explore.

The principles underlying this account, namely modularity, developmental architecture, and preservation, may not be confined to fungi. Bridging micro- and macroevolutionary scales remains an active programme (Rolland et al., 2023), and the framework offers a design that travels. Whatever the clade, the test asks for the module underlying a lost trait, for that module's preservation state against an ancestral baseline, and for the reversibility record against which the association is judged. Similar principles recur across the developmental systems of plants, animals, and microbes. Whether the framework can illuminate their evolution is an open question, but one we think worth

pursuing. The central variable has not yet been measured; the tools needed to measure it, however, are increasingly within reach. The path forward is uncertain, but it is open, and we hope this framework invites others to take it up.

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Conflicts of Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, I used GLM-5.3 (Zhipu AI, Beijing Zhipu Huazhang Technology Co., Ltd., Beijing, China) to help me review the manuscript for grammar errors, correct them, and polish the language. The AI tool did not generate, modify, or interpret any scientific content, arguments, or conclusions. I reviewed all AI-assisted outputs against the original scientific content to ensure accuracy and consistency, and I take full responsibility for the content of the publication.

Table 1 | Expectations, grades, and status.

Expectation	Grade	Required observation	Current status
E1 — module integrity ranks lineages on the reversibility spectrum	Core; testable in principle	Independent integrity scores associate with reversal probability across homologous frameworks, within strata of matched loss-age and ecology (Section V)	Construct unmeasured; association untested; stratification designs specified
E2 — nonlinear decline of reversal probability	Core; conceptual	Threshold models outperform linear alternatives near the critical zone	Qualitative expectation only; parameters undetermined
E3 — regulatory decay precedes structural decay	Core corollary; conceptual	Earliest relaxation signals localized to the bottleneck core and its regulatory connections; ordinal null model with susceptibility covariates (Section V)	Corollary of the framework's logic; null model specified; empirically grounded null distribution not yet constructed
E4 — HGT functional categories differ between integrity regimes	Peripheral; testable in part	Acquired genes, classified by the module they compensate, segregate with integrity regime	Partial support (Liu et al., 2025); the functional-category test itself not yet performed
E5 —	Core;	Elevated dN/dS,	Existence case (Mead

Expectation	Grade	Required observation	Current status
bottleneck-core degradation signatures in locked lineages	testable in principle	pseudogenization enrichment, and co-expression disintegration at the cores of locked but not flexible lineages	et al., 2020); mycorrhizal gradient offers a candidate contrast design (Supplementary Box 2, case 4); systematic contrasts untested
E6 — reversal probability may vary with architectural depth	Core-adjacent; testable in principle	Clade-level pattern: reversal frequency tracks per-clade estimates of architectural depth (Section II, grain convention)	High-reversibility end broadly consistent with evidence (Thomé et al., 2024); gradient unquantified
E7 — somatic channel efficiency	Peripheral; prospective	Greater morphological disparity at comparable integrity in clonal, long-lived network lineages	Unquantified; contribution across lineages awaits assessment
E8 — lateral transitions among parasitic modes involve modular changes	Peripheral; conceptual	Host jumps among parasitic modes accompanied by fewer, more modular genomic changes than transitions to saprotrophy	Convergent-signature support; transition-count prediction unquantified

Table 2 | Glossary of technical terms. Terms marked with an asterisk (*) are introduced by this paper or given a specific sense here; unmarked terms are established usages, with the sense intended in this paper made explicit. Entries are compressed for lookup; the sections indicated carry the full definitions and their argument. Notation (dN/dS) is filed alphabetically, ignoring case.

Term	Definition
Ancestral state reconstruction	Statistical inference of character states at internal phylogeny nodes; supplies the baseline from which module integrity is measured (§II).
Architectural depth*	The structural complexity of the ancestral module: number of regulatory layers, integration among components, size of the target sets commanded by cores; a property of the reference state, distinct from present preservation (§IV(3)).
Attractor landscape (basin)	In dynamical descriptions of gene regulation, the set of stable expression states of a network, each a basin that attracts nearby states; used here as heuristic for morphological accessibility (§IV(2)).
Ballistosporic	Forcibly discharging spores from an exposed hymenophore; the discharge mode lost in gasteroid transitions (§I).
Bottleneck core*	A top-level regulatory node whose loss prevents coordinated activation of the downstream module, for which no paralogous substitute exists, and on which any compensatory pathway depends (§II).
Canalization	Buffering of developmental output against genetic and environmental perturbation, such that some mutations never reach phenotype (§III(1)).
Character identity network (ChIN)	Wagner's term for the regulatory core that specifies what a character is and maintains its historical continuity across realizations (§II).
Chytrid	Member of the earliest-diverging fungal lineages, typically aquatic, many flagellated; the framework's outgroup contrast (§VI; Supplementary Box 2, case 9).
Congruence class	The set of diversification histories statistically indistinguishable, on an extant timetree, from a given inferred rate set; the core identifiability limit of diversification models (Supplementary Box 3).
Constructive neutral evolution (CNE)	Accumulation of structural complexity through fixation of mutations neutral in isolation that create new interdependencies, without positive selection (§III(2)).

Term	Definition
Co-option	Evolutionary redeployment of an existing developmental module into a new functional or structural context (§III(1)).
Critical zone*	The region of declining module integrity across which reversal probability falls at an accelerating rate; a conceptual region, not a sharply locatable threshold (§IV(3)).
Dependence trap	In symbiont genome reduction, the exchange of short-term ecological gain for long-term evolved dependence on the host (Supplementary Box 2, case 5).
Developmental constraints	Biases on the production of variant phenotypes imposed by the structure, composition, and dynamics of the developmental system (§II).
Dikarya	The fungal subkingdom containing Ascomycota and Basidiomycota; the framework's principal domain (§VI).
Distribution of fitness effects (DFE)	The probability distribution of fitness effects of new mutations; its low-effect fraction estimates plateau width (Supplementary Box 3).
Diversity-generating pathway*	The four-process machinery — variation supply, quasi-neutral retention, somatic expansion, selective recycling — that sets the breadth of exploration; its determinants differ from those maintaining module integrity (§III).
dN/dS	The ratio of non-synonymous to synonymous substitution rates; elevation signals relaxed or positive selection and serves here as a degradation signature (§V; Supplementary Box 1).
Dollo's law	The generalization that a complex trait, once lost, is not regained; historically descriptive, now the object of mechanistic testing (§I).
Effective population size (N_e)	The size of an idealized population experiencing the same drift as the observed one; level-dependent in modular, partially clonal organisms (§III(3)).
Evolvability	The capacity to generate heritable phenotypic variation (§III(1), §VI).

Term	Definition
Forward and return accessibility*	The two directions of the constraints question: why some variants are never produced (forward), and why some, once lost, cannot readily be produced again (return) (§I, §II).
Gasteroid	Bearing a closed fruiting body in which spores mature internally, without active discharge (§I; Supplementary Box 2, case 2).
Gene regulatory module (GRM)*	The network that encodes and executes a specific biological function or developmental process; the framework's unit of analysis (§II).
Genet and ramet	The genetic individual founded by one zygote (genet) and its clonal, potentially independent subunits (ramets) (§III(3)).
Genetic–phenotypic decoupling*	The failure of genetic change to reach phenotypic output, or of genetic or phenotypic change to reach fitness consequences; organized here into an architectural layer (§III(1)) and a populational layer (§III(2)); descriptive rather than a measured construct (§I, §III).
Grain convention*	The rule that predictions are formulated at the module level; composite and ensemble traits are aggregated explicitly rather than attributed to a single module (§II).
Heterokaryosis	The coexistence of genetically distinct nuclei within one mycelial cytoplasm (§III(3)).
Horizontal gene transfer (HGT)	Acquisition of genetic material across species boundaries rather than by descent; the external route of module compensation (§III(4), §V, §VI).
Hymenophore	The spore-bearing surface of a fruiting body — gills, pores, teeth, and related forms (§I; Supplementary Box 2, case 1).
Kernel	In the Davidson-Erwin GRN hierarchy, a deeply conserved, densely connected subcircuit whose modification is held to yield developmentally irreversible shifts (§II).
Latent variable	An unobserved quantity inferred statistically from observed indicators; module integrity is modelled as

Term	Definition
	one (Supplementary Boxes 1, 3).
Mating-type locus	The genomic region determining mating compatibility in fungi, functioning in many lineages as a single-locus developmental switch whose loss collapses the sexual cycle (§II).
Module integrity*	The systemic preservation state of a GRM relative to its ancestral baseline; continuous, inferred from genic content, network topology, and expression response (§II; Supplementary Box 1).
Most recent common ancestor (MRCA)	The latest ancestor shared by the lineages under comparison; the reference node for integrity baselines (§II).
Muller's ratchet	The irreversible accumulation of deleterious mutations in populations lacking recombination (Supplementary Box 2, case 5).
Mycorrhizal symbioses (ECM, ericoid, orchid, AM)	The four major plant-fungal mutualisms: ectomycorrhizal (root-sheath, intercellular; mainly Dikarya), ericoid and orchid (intracellular, with broadly retained saprotrophic capacity), and arbuscular (ancient, obligate, with extensively reduced biosynthetic repertoires) (§I; Supplementary Box 2, case 4).
Neutral network	A connected set of fitness-equivalent genotypes through which populations drift without change in fitness (§III(2), §IV(2)).
Non-compensability*	The defining property of a bottleneck core: absence of any alternative route to the module's functional output once the node is lost; historically contingent and evolvable (§II, §IV(2)).
Ortholog and paralog	Genes related by speciation (orthologs) or by duplication within a lineage (paralogs); standard integrity comparisons require confirmed orthology (§II; Supplementary Box 1).
Paralichen	Goward's term for a loosely organized fungal-algal association that provides a relational interface without the integrated thallus of a true lichen; a candidate low-threshold route to re-lichenization (Supplementary Box 2, case 6).

Term	Definition
PCWDE	Plant-cell-wall-degrading enzyme (cellulases, pectinases, and relatives); erosion of PCWDE repertoires tracks metabolic-module degradation across mycorrhizal transitions (Supplementary Box 2, case 4).
Phialide	The flask-shaped cell that produces asexual spores in many filamentous fungi; recurrent loss of its regulator *abaA* is Expectation 5's existence case (§V).
Phylogenetic logistic regression	Logistic regression of a binary outcome that corrects for phylogenetic non-independence among taxa (Supplementary Box 3).
Pseudogenization	The accumulation of disabling mutations that renders a gene non-functional while it remains detectable in the genome; the cumulative component of sequence degradation (§II; Supplementary Box 1).
Quasi-neutral fitness plateau*	The fitness-effect region within which selection cannot discriminate among variants; the material of future innovation and, at the same time, the entry channel of bottleneck-core erosion (§III(2)).
Regeneration stage and sorting stage*	The two ordered stages of any reversal: the developmental system must regenerate the lost variant, which must then survive selection and drift to fixation (§I).
Reversibility spectrum*	The continuous range of trait transitions, from freely bidirectional to return paths that are statistically closed (§I).
Saprotroph, biotroph, and necrotroph	Nutritional modes: feeding on dead organic matter; feeding on living host tissue without killing it; killing host tissue and then feeding on it (§I, §IV; Supplementary Box 2, case 5).
Secotioid	A partially closed fruiting body intermediate between agaricoid (open, gilled) and gasteroid (closed) forms (§IV(3); Supplementary Box 2, case 2).
Selective recycling*	The acquisition of adaptive value by a trait previously maintained under drift, followed by

Term	Definition
	directional refinement; exogenous and endogenous routes exist (§III(4)).
Somatic evolutionary channel*	Generation, spread, and local fixation of heritable variation within a single mycelial network through nuclear migration and hyphal fusion, without meiosis (§III(3)).
SSE models (state-dependent speciation and extinction; MuSSE)	Phylogenetic models linking character states to speciation, extinction, and transition rates; used here for transition rates, under known identifiability limits (Supplementary Box 3).
Statistical inaccessibility*	The state of a lost trait whose return would require independent mutational events whose joint probability has fallen to an extremely low level; used throughout in this joint-probability sense (§IV(1)).
Threshold model	The comparative model treating a binary character as the expression of a continuous latent variable crossing a threshold (§V; Supplementary Box 3).
Zero-force expectation	The proposition that diversity and complexity tend to increase in evolutionary systems in the absence of opposing forces (McShea & Brandon, 2010) (§IV(1)).

Supplementary Box 1 | Approximating module integrity: dimensions, identification, and caveats

Inference of module integrity requires joint assessment from at least three operational dimensions, each corresponding to a different level of observable evidence. The evaluation scale for each dimension is the ancestral-node baseline described in the main text, rather than any extant species. When paralogous modules have arisen through lineage-specific duplication and expansion, comparing them with the ancestral orthologous module requires verification that each post-duplication copy retains a

regulatory target profile equivalent to the ancestor's. The regulatory target profile is the set of target genes directly regulated by a given node and all their regulatory relationships. If subfunctionalization or neofunctionalization has occurred after duplication, that copy should not be directly included in the integrity comparison with the original orthologous module.

(1) Genic content. By combining homology searches with annotation pipelines, we can quantify the retention and loss patterns of core orthogroups relative to the ancestral node and score the pseudogenization rate of genes within the module, which captures the cumulative component of sequence degradation. This dimension therefore assesses how much of the ancestral module has been retained and how functionally intact the remaining components appear to be.

(2) Network topology. Based on cross-species transcriptomic data, co-expression network construction can be used to reconstruct the regulatory network structure within the module; topological comparison with an inferred ancestral regulatory network would then focus on the degree of retention of upstream and downstream targets of the bottleneck core and on the pattern of missing regulatory connections. As specified, this dimension measures retention, that is, the degree to which ancestral connections are preserved rather than lost. A complementary gain component is its natural extension: regulatory connections to elements absent from the ancestral module whose removal collapses module output. As with hub status, gained connections cannot be read as load-bearing from co-expression alone; confirmation requires perturbation or cross-lineage convergence filtering, and the identification path mirrors the bottleneck-core protocol with the sign reversed. The gain component defines a separate quantity, entrenchment load, rather than a fourth manifest variable of the integrity model: retention and construction are measured apart, so that constructive pathway work does not erode

integrity by definition.

(3) Expression response. Under standardized free-living induction conditions, differential expression and pattern-recognition methods can be used to measure the transcriptional response patterns of module genes, and their similarity to the reconstructed ancestral expression profile computed by cosine similarity or Euclidean distance. This dimension assesses whether the remaining components can still work together in an activated state, and how far their degree of coordination lies from the ancestral state.

Three evolutionary situations should be distinguished in applying such a design. (Case A) When module composition is relatively conserved and gene content is dominated by orthologous relationships, the multi-dimensional inference above is the standard applicable scenario. (Case B) When lineage-specific duplication, expansion, and subfunctionalization make orthologous relationships difficult to establish, the genic-content dimension should be adjusted from ortholog-group retention ratio to coverage of functional equivalents for the core functional set: whether every core function performed by the ancestral module has at least one copy in the current gene pool capable of performing it. The topology dimension should shift from edge-to-edge comparison to assessment of the conservation of regulatory relationship topology among functional units; the expression-response dimension can remain unchanged. (Case C) When a module has undergone deep degradation and later re-acquired a similar function with a fundamentally replaced molecular basis, similarity at the phenotypic level does not in itself indicate homology of the modules. Such cases belong to the separate topic of functional convergence rather than to direct comparison under this framework.

Data from the three dimensions can be integrated through a Bayesian latent-variable model, offered as a schematic rather than a fitted proposal. These dimensions capture

the module's state at several levels, including presence, connectivity, and execution; joint inference would exploit the fact that they evolve asynchronously. The observed multi-source evidence serves as manifest variables; module integrity is inferred as the latent quantity; posterior distributions quantify estimation uncertainty. The phylogenetic covariance structure enters as a random effect on lineages, so that the inference reflects lineage-specific relative decline rather than the spurious correlations of shared evolutionary history. Under incomplete data, conservative homologous gene sets and comparable transcriptomic data can support relative comparisons against the same ancestral baseline, and experimentally accessible proxies for a module's main functions can provide a check on the inferred degradation rankings. A fully specified implementation, including likelihood forms, priors, identifiability conditions among others, belongs to the research agenda.

Identifying bottleneck cores. In experimentally tractable species, identification may proceed along the following path: construct gene co-expression networks and identify candidate nodes occupying hub positions (highest degree or betweenness centrality); perform loss-of-function experiments on candidate hub genes to assess their effects on key functional outputs; and use transcriptomic profiling after perturbation to detect whether the module's gene set shows a coordinated decline in expression associated with biological phenotypic abnormalities. A final step assesses, through genome-wide paralog searches and functional redundancy analyses, whether other genes could maintain the node's essential function at the level of protein interaction interfaces and regulatory target profiles. If none are currently detectable, the key regulatory node may be judged a bottleneck core. In cross-lineage comparisons, orthologous candidate nodes can be compared directly within an ancestral-derived framework. Paralogous copies require verification that they have acquired a regulatory target profile equivalent to that

of the ancestral orthologous node; a copy failing this verification should not be recognized as occupying the same topological position. In non-model species, where experiments are infeasible, molecular evolutionary signals may provide indirect evidence. By comparing genomic data from lineages that retain the trait with those from lineages that have lost it, we can identify the genes that experienced the earliest relaxation of selection after trait loss. Such relaxation may be reflected in elevated dN/dS ratios, enrichment of premature stop codons, and disintegration of co-expression networks. Convergent signals across independent trait-loss events may help distinguish bottleneck cores from genes undergoing random drift. Expectation 3 specifies that we should assess convergence at the level of regulatory position, using evidence from conserved cis-regulatory motifs, synteny, and shared downstream targets, rather than from gene identity alone. The validity of such analyses presupposes, as far as possible, that the genes compared are orthologous; these analyses are in principle feasible with the large amount of publicly available fungal genomic and transcriptomic data. Entrenchment identification mirrors the protocol above with the sign reversed: identify regulatory connections to elements absent from the ancestral module, perturb the candidate entrenching partner, and ask whether loss of the node is deleterious in the current background but tolerable in the partner-removed background. Where perturbation is infeasible, convergence filtering across independent lineages that gained the same dependency can substitute.

Methodological caveats. Several difficulties, arising from the tension between molecular biology and evolutionary biology, accompany any cross-lineage application of this design. First, cross-species omics data are systematically heterogeneous in their generation conditions, sampling time points, tissues, developmental stages, and induction conditions, and such technical variation may obscure true biological signals.

In addition, the transient transcriptional perturbation captured by loss-of-function experiments and the gradual relaxation of selection accompanying bottleneck-core degradation over evolutionary time belong to different causal levels. The former reflects the immediate robustness of a steady-state network within a single generation; the latter records the process of mutation fixation accumulated over many generations. The quantitative mapping between the two currently lacks systematic theoretical derivation. Second, the statistical covariation represented by co-expression networks is inherently disconnected from regulatory causality. In non-model species, computationally predicted hubs may merely be passenger genes driven by shared signals, so hub status alone should not be equated with a bottleneck core; resolution usually requires direct testing through loss-of-function experiments or cross-filtering of convergent mutational signals across independent lineages. Third, causal direction is difficult to establish. Inactivation of a bottleneck core and degradation of downstream genes may be mutually causal in evolution, forming a positive feedback loop: attenuation of the regulatory node releases selective constraints on its targets, while pseudogenization of targets may in turn eliminate the functional demand that maintained selective pressure on the node. In the absence of precise ancestral state reconstruction and time-series genomic data, causal direction therefore retains inherent uncertainty. Fourth, a judgment of "no compensation" can only be provisional: conventional paralog searches are limited by annotation completeness, and compensation may involve non-orthologous, convergently evolved genes whose sequence divergence is sufficient to evade homology-based detection. Judgments should therefore be formulated as "no equivalent compensation detected within the scope of current data and analytical methods," and constrained by strict functional-redundancy criteria and evolutionary-age weighting. Compensatory scarcity is more

likely to be assessed accurately in recently diverged lineages; in deeply diverged ancient clades, the prior probability of convergent evolution of alternative pathways increases with time. These challenges are not unique to this framework; they are the general structural dilemma of evolutionary developmental biology in bridging regulatory network perturbation (transient) and trait transition (diachronic). Correlational noise, falsification of redundancy, and causal attribution cannot be fully bypassed under the current paradigm. Specific technical routes and statistical thresholds depend on the genetic tractability and data scale of the study system, and belong to the lineage-specific research agenda.

Extension strategies for ambiguous homology. Where orthologous relationships are difficult to establish, three complementary strategies may serve as auxiliary supplements: functional homology groups defined by cis-regulatory motif and target-profile similarity; structural equivalence comparisons based on regulatory network topological fingerprints; and functional equivalence assessment based on dynamic response characteristics under standardized perturbation (multi-time-point transcriptomic curves). The first of these depends heavily on the quality of regulatory annotation in the reference species, and motif degeneracy may introduce false positives in genome-wide scans; the second is constrained by differences in network inference quality among non-model species; the third requires multi-time-point transcriptomic data under strictly standardized conditions covering the complete response process. In principle, outputs of each strategy should be compared only within that strategy. When the homology premise of the standard framework is satisfied, inferences based on the homologous framework should prevail; when homologous relationships are incomplete or unavailable, the above strategies serve as auxiliary supplements.

For the analyses designed here, we regarded the following tools and algorithms as

potentially suitable. Genic content: orthology inference via OrthoFinder with a custom ortholog database (Emms & Kelly, 2019); pseudogene identification and loss-of-function variant annotation via PseudoPipe or SnpEff (Zhang et al., 2006; Cingolani et al., 2012). Network topology: co-expression network construction via WGCNA soft-thresholding correlation or GENIE3 decision-tree-based inference (Langfelder & Horvath, 2008; Huynh-Thu et al., 2010). Expression response: DESeq2 combined with principal component analysis or gene set enrichment analysis (Love, Huber & Anders, 2014). Ambiguous-homology extensions: FIMO/TOMTOM scans with Jaccard similarity or hypergeometric tests of target-set overlap (Grant, Bailey & Noble, 2011; Gupta et al., 2007); FANMOD or WaRSwap subgraph-frequency distributions (Wernicke & Rasche, 2006; Megraw, Mukherjee & Ohler, 2013); multi-time-point transcriptomic curves following the logic of methods such as DYNAMO (Nudelman et al., 2021).

Supplementary Box 2 | The framework across the fungal kingdom

The following cases illustrate how the framework might interface with known evolutionary patterns in major fungal groups. They show how the framework could organize existing observations and where future tests could be conducted. Each case differs in evidence strength, in its point of contact with the core mechanism, and in the confidence of its inferences. They are offered as potential heuristic extensions and as

possible bases for empirical case studies; the framework may provide a unified organizational logic for these scattered observations and suggest directions for future empirical testing.

(1) Fruiting bodies and hymenophores: a spectrum of directional constraints

Transitions involving peripheral nodes or non-bottleneck regulatory genes with paralogous compensation might appear frequently reversible in phylogenies, because the corresponding attractor basins would remain shallow and drift could move the system bidirectionally. Transitions whose realization depends on the loss of bottleneck cores should instead show strong asymmetry, since reversal would require rebuilding a coordination structure that has already disintegrated. Different transitions might therefore occupy different positions on a spectrum of directional constraint rather than falling into two discrete categories.

Fruiting body configuration and hymenophore morphology show roughly this graded pattern within Agaricomycetes. At one pole, transitions among open hymenophore types appear frequent and bidirectional. Gills, pores, and teeth have each evolved convergently in at least eight orders of Agaricomycetes (Varga et al., 2019), and transitions between poroid and gilled hymenophores occur even between closely related sister taxa.

Lamellate genera such as Hygrophoropsis, Paxillus, and Phylloporus are nested within predominantly poroid Boletales, while poroid genera such as Favolaschia are nested within gilled clades of Agaricales (Virágh et al., 2022).

Directionality exists in places: within Lentinus and its relatives, parsimony-based ancestral state reconstruction recovers a pore-bearing ancestor with multiple independent transitions to angular pores and lamellae, while the reverse transitions

appear less frequent (Sathiya Seelan et al., 2015). Neither direction, however, seems completely closed.

At a broader scale, maximum-likelihood reconstructions across the homobasidiomycetes find the transition rate from simple to complex forms exceeding the reverse rate three- to six-fold, with a null model of symmetric rates rejected (Hibbett & Binder, 2002).

The observed spectrum is compatible with the framework's core logic. Transitions among open hymenophore types plausibly involve peripheral nodes whose module cores remain intact, so the attractor basins stay shallow and the transitions appear frequently reversible in phylogenies. The gasteroid case, examined next, is the extreme and best-quantified position on this spectrum.

A mechanistic reading of the homoplasy is available: hymenophoral ontogeny, including pores, teeth, gills, and labyrinthine forms, may arise through self-organized pattern formation, so that distantly related lineages converge on the same fertile-surface morphologies (Kuhar et al., 2022). Convergence here may reflect limited options generated by a conserved developmental grammar, rather than repeated adaptive optimization to a single global optimum.

The regulatory-hierarchy interpretation of the hymenophore spectrum remains a hypothesis, the specific genes and regulatory architectures underlying hymenophore type transitions have not yet been mapped. The framework's reading, that bidirectional transitions involve peripheral nodes and directional transitions involve bottleneck cores, awaits direct testing through the module-specific localization prediction (Expectation 3).

(2) Gasteroidization as developmental truncation

If gasteroidization truncates the developmental program at a specific module node, losing or silencing a bottleneck core that controls late-stage fruiting-body maturation, then several patterns might be observable: the forward transition should occur frequently while the reverse transition would be statistically near zero; the initial truncation could be genetically simple, because a single bottleneck-core mutation might disable an entire downstream module; the initial state might be ecologically permissive, because the truncated morphology could still satisfy basic spore-dispersal requirements; and a temporal gap might exist between initial allelic polymorphism and phylogenetic lock-in, reflecting the time required for downstream regulatory connections to degrade after the bottleneck core is lost.

The expectations appear to find some support in the best-quantified pattern in fungal macroevolution.

Molecular phylogenetics first established that gasteroid morphologies are polyphyletic, having evolved at least four times independently within the homobasidiomycetes from ancestors with exposed hymenophores (Hibbett et al., 1997). Each such transition is repeatedly associated with loss of active spore discharge. Later analyses moved the pattern from demonstration to quantification.

On a megaphylogeny of 8,400 Agaricomycetes species, 462 morphological transitions were inferred, including 123 independent origins of gasteroid forms; the reverse transition was found highly unlikely, though statistically not strictly rejectable (Sánchez-García et al., 2020; Varga et al., 2019).

Direct statistical tests of irreversibility yielded comparable results: under both binary and five-state coding, the hypothesis that gasteroid evolution is irreversible could not be rejected (Hibbett, 2004). Diversification analyses add a further layer: in several

clades representing independent origins of the habit, gasteroid lineages appear "trapped" in the closed state, with most optimal models assigning them higher net diversification rates than their non-gasteroid ancestors. Their low global frequency (~8.4% of Agaricomycetes) may therefore be better explained by the recent origin of most gasteroid lineages than by poor diversification (Wilson et al., 2011).

The truncation reading finds a concrete developmental substrate. Fruiting-body development runs as quasi-independent subroutines whose modification or omission can produce drastically altered morphologies (Kües & Navarro-González, 2015), and the cleanest existence proof comes from Lentinus tigrinus, in which agaricoid and secotioid fruiting bodies occur under the same genetic background. Bulk-segregant analysis combined with differential expression narrowed the candidates to roughly 25 fruiting-body development genes spanning ~1.5 Mb (≈ 4% of the genome); the gasteroid trait behaves as a recessive allele at a single locus, and the differentially expressed set comprises only 5-7% of the transcriptome (Wu et al., 2018). This is an existence proof, not a demonstration of generality.

Two further observations are compatible with the framework's reading of how such a state might become fixed. First, gasteroidization occurs in diverse ecological settings, and no single adaptive narrative seems sufficient on its own; the classical secotioid syndrome, for example, links closed forms to drought-prone environments (Thiers, 1984). The framework could treat such adaptive readings as complementary accounts of the intermediate stage rather than as competing explanations, since the initial fitness cost of truncation might be low enough to be retained in multiple ecological contexts. Second, the coexistence of gasteroid and agaricoid morphologies within single populations suggests the predicted temporal gap between allelic polymorphism at the

individual level and evolutionary locking at the phylogenetic level. Reversal might be relatively possible in the initial stage, but as bottleneck cores are lost and downstream regulatory connections degrade, the threshold for reversal could continue to rise.

The same logic need not depend on the special morphology of an enclosed hymenophore. Secondary smooth hymenophores provide a heuristic extension: stipitate stereoid forms originated independently multiple times and sit at the tips of the tree (Sjökvist et al., 2012), repeated smooth-hymenophore types occur within Marasmius (Oliveira et al., 2024), and directional tendencies exist even among open hymenophore types (Sathiya Seelan et al., 2015). Constructing a single adaptive narrative for this simplified morphology is difficult. The framework offers a possible internally consistent explanation. If the secondary production of smooth hymenophores involves truncation or silencing of the modules that build complex hymenophores, and the altered developmental state can still satisfy basic spore-dispersal requirements, then the repeated independent origins and the asymmetric lack of reversal might be explainable by the same truncation logic.

Developmental genetic or comparative genomic studies of smooth hymenophores remain insufficient, and the causal association with the framework's mechanism awaits future testing in suitable groups with genomic resources. The truncation model of gasteroidization, while plausible and supported by the Lentinus tigrinus case, has not yet been tested at the level of specific bottleneck cores.

(3) Yeast-like transition and dimorphism: an experimentally tractable testing ground

The framework's core prediction is that evolutionary reversibility may be governed by the regulatory hierarchy of the genes involved, with bottleneck-core loss potentially

producing directional constraint and peripheral-node changes allowing bidirectional transitions. Testing this prediction requires systems in which the genetic architecture of a developmental transition can be experimentally manipulated, and dimorphic fungi appear to offer such a system: the yeast-hypha transition is genetically tractable in multiple lineages, reversible switching can be observed directly in the laboratory, and the molecular machinery underlying the transition has been characterized to varying degrees across independent origins of dimorphism.

Dimorphism has appeared independently in multiple lineages, providing natural replicates of the same developmental transition (Nagy et al., 2014). Phylogenetic analysis of Ophiostomataceae suggests that dimorphism is likely ancestral in the family, that its subsequent evolution shows a strong phylogenetic signal, and that its retention and loss may be associated with an ambrosia lifestyle (Abdrabo et al., 2026).

The molecular machinery is increasingly well resolved: in pathogenic dimorphic fungi, switching is triggered by environmental signals, and the regulatory module connecting the two terminal states has been characterized in several systems (Boyce & Andrianopoulos, 2015; Zhao & Lin, 2021). A conserved regulator appears to govern morphology across Saccharomyces, Candida, and Histoplasma despite lineage-specific target sets (Cain et al., 2012).

In Candida albicans, a single-nucleotide change in SSN3, a subunit of the Mediator complex, sufficed to bypass the Efg1/Cph1-dependent filamentation program and restore filamentation in a nonfilamentous mutant (Wartenberg et al., 2014). That observation probes how far a developmental switch is genuinely bottlenecked, and appears consistent with the evolvable non-compensability discussed in Section IV(2).

Reversible switching is not merely a laboratory artifact: white-opaque switching has

been observed in natural mating-competent isolates under host-simulating conditions (Xie et al., 2013), and trimorphic intermediate states might serve as stepping stones in morphospace (Bastidas & Heitman, 2009).

Dimorphic systems make a direct test feasible by providing tools such as knockouts, regulatory rewiring, and comparative genomics of closely related dimorphic and monomorphic species. Where the transition to obligate yeast growth involved the loss of a bottleneck core, reversal to hyphal growth would be statistically unavailable; where the loss involved peripheral nodes, or compensatory regulatory rewiring remains possible, reversal might be possible under appropriate genetic or environmental conditions.

Phylogenetically, the transition from a hyphal or dimorphic ancestor to an obligate yeast state has generally been considered irreversible, whereas clear cases of obligately hyphal forms reacquiring stable facultative dimorphism appear to be lacking. This pattern seems consistent with the framework's expectation of directional constraint arising after bottleneck-core loss, but the current evidence does not yet appear strong enough to support more definite conclusions. The number of dimorphic systems with sufficiently detailed molecular characterization remains small, and systematic comparisons across multiple independent origins of dimorphism have yet to be performed. That design would most directly test the framework's predictions.

(4) The mycorrhizal gradient

The framework would rank mycorrhizal types by the predicted reversibility of the transition to saprotrophy, based on the aggregate preservation state of the free-living metabolic modules involved. Ectomycorrhizal (ECM) fungi might show intermediate retention of lignocellulose-degrading modules and intermediate reversal probabilities;

ericoid and orchid mycorrhizal fungi could show higher retention and higher reversal probabilities; and arbuscular mycorrhizal (AM) fungi would show the deepest metabolic degradation and near-zero reversal probabilities. The framework further expects that integrity loss in these symbioses should be module-specific rather than genome-wide, concentrated in genes whose functions are rendered redundant by host provisioning while genes serving other functions would remain under purifying selection.

The genomic correlates of this gradient appear correspondingly graded. Hibbett et al. (2000) provided the classic demonstration that ECM symbiosis is evolutionarily labile, repeatedly gained from saprotrophic ancestors and lost again, with multiple reversions to free-living states. Counts of independent origins have reinforced the demonstration: roughly 60 (Martin et al., 2016) or, in the most permissive estimates, 78-82 across 251-256 genera (Tedersoo & Smith, 2013). Across 13 mycorrhizal and 5 saprotrophic genomes, ECM fungi show reduced plant-cell-wall-degrading enzyme (PCWDE) complements relative to wood-decay ancestors, yet retain a distinct set of PCWDEs whose identity differs among lineages; 7-38% of symbiosis-induced genes are orphans encoding secreted effector-like proteins (Kohler et al., 2015; Eastwood et al., 2011; Floudas et al., 2012). A phylogenomic analysis of 135 fungal genomes confirmed similar patterns at larger scale: widespread PCWDE loss, co-option of ancestral saprotrophic genes for symbiotic roles, and diversification of new symbiosis-induced genes (Miyauchi et al., 2020). Genome sequencing of additional ECM lineages, such as the dominant symbiotic fungus Cenococcum geophilum, has reinforced this picture of lineage-specific retention (Peter et al., 2016).

A marked molecular case sits at the interface of the gradient. In Amanita, ECM symbiosis appears to have a single origin, and the transition coincided with loss of two

cellulase genes, an endoglucanase and a cellobiohydrolase; growth experiments confirm that symbiotic species cannot grow on complex organic matter (Wolfe et al., 2012). A third, late-acting cellulase is retained in many but not all species, illustrating that the erosion may be graded even within one lineage. Even within a single genus the genomic correlates differ: transposable-element dynamics distinguish asymbiotic from ectomycorrhizal Amanita species (Hess et al., 2014).

Comparative genomics of 21 symbiotic Boletales extends the point: PCWDE repertoires are reduced but lineage-specific, lignocellulose oxidoreductases are retained at levels similar to brown-rot species, and transposon proliferation drives elevated evolution of effector-like secreted proteins (Wu et al., 2022; Looney et al., 2022). A preprint survey of 182 genomes further distinguishes gene loss from relaxed selection, finding specialized cellulases and pectinases under significantly relaxed selection in ECM fungi while a multifunctional core family remains under purifying selection (MinSeo & Shin, 2026).

At the high-integrity end of the series, ericoid mycorrhizal fungi retain gene repertoires close to those of saprotrophs and pathogens, with both saprotrophic and symbiotic capacities seemingly intact (Martino et al., 2018). The orchid mycorrhizal model Tulasnella calospora retains a broad carbohydrate-active enzyme arsenal whose expression is finely regulated between saprotrophic growth and symbiosis (Adamo et al., 2020).

The orchid case also raises a side question worth flagging. Many mycoheterotrophic plants draw carbon and nutrients from their fungal partners, sometimes extensively, yet appear to do so without elaborate machinery for suppressing or manipulating the fungus, unlike many other host-parasite systems where the parasite must overcome active host defenses. One possible reading is that the fungal partner is not strongly selected to resist.

Many orchid mycorrhizal fungi are facultatively saprotrophic or associate with multiple photosynthetic plants, so the carbon drain imposed by a mycoheterotrophic partner may be buffered by alternative carbon sources. The plant's draw need not then impose strong selection on the fungal carbon-acquisition machinery, and the fungus may tolerate the relationship without dedicated, high-intensity defenses or a defensive arms race. Whether this asymmetry in carbon-source breadth explains the apparent ease of mycoheterotrophy in fungus-associated plants remains open.

The observed pattern is broadly consistent with the rank-ordering prediction: intermediate retention in ECM fungi, higher in ericoid and orchid mycorrhizal fungi, deepest in AM fungi. The degradation is also module-specific, concentrated in PCWDEs while core metabolic and mycelial development genes are retained, and the Amanita case illustrates the graded nature of integrity loss within a single lineage. The ECM habit itself occupies a continuum between biotrophy and saprotrophy rather than a discrete category (Plett & Martin, 2011).

The robustness of all these reversal inferences depends on the assumptions of the phylogenetic comparative models used: with 2,174 taxa and five markers, conclusions about ECM-non-ECM transition rates change substantially depending on whether variable rates are permitted (Sheikh et al., 2022). These signals should therefore be understood as phylogenetic inferences awaiting further clarification, not as settled measurements of a spectrum position. Nor does the ECM transition uniformly raise diversification rates in the Tricholomatineae, consistent with its treatment here as an intermediate-integrity state rather than a key innovation (Sánchez-García & Matheny, 2016).

(5) Parasitic strategies and the critical zone

Obligate parasitism might represent the extreme low-integrity endpoint of the framework's spectrum: sustained host provisioning could render entire metabolic modules redundant, selection on the bottleneck cores controlling those modules would relax, and downstream gene loss would follow; directional constraint would then emerge as integrity falls below a critical zone. The framework also bears on the stable intermediate strategies that occupy this spectrum. Facultative parasitism and necrotrophy, which persist over evolutionary timescales, may be read as lineages that have entered the constraint regime but have not yet crossed the critical zone far enough to generate strong directionality. Their stability could be explained by antagonistic pleiotropy, with the same genes carrying opposite fitness effects across free-living and parasitic contexts so that selection tends to cancel, together with the quasi-neutral fitness plateau, which allows both capacities to persist without strong directional pressure. Where integrity is held in such a near-neutral balance, the constrained regime is entered only slowly, if at all.

The large-scale documentation of module erosion in obligate parasites is clear. Reconstruction of the ancestral gene repertoire of Pneumocystis jirovecii indicates that 2,324 genes were lost in acquiring obligate biotrophy (Cissé et al., 2014). The losses concentrate in amino-acid and thiamine biosynthesis, inorganic nitrogen and sulfur assimilation, and purine catabolism, precisely the pathways rendered redundant by host provisioning, together with the RNAi machinery and many lytic proteases. Powdery mildews show a similar combination of erosion and innovation in different genomic compartments: massive retrotransposon proliferation and loss of primary and secondary metabolic enzymes alongside an effector repertoire of which fewer than 10% of candidate genes are conserved across three species (Spanu et al., 2010). Rust fungi similarly combine extensive gene loss with lineage-specific expansions (Duplessis et

al., 2011). At the regulatory level, obligate host-associated pathogens show global shrinkage of transcription-factor repertoires (Möller & Stukenbrock, 2017). The endpoint of this trajectory is exemplified by microsporidia, whose severe reductive evolution pervades cell structure, metabolism, and genome (Keeling & Fast, 2002), reaching an 8.5-Mb genome in Trachipleistophora hominis (Heinz et al., 2012). Ongoing genome decay is explained in part by Muller's ratchet operating on non-recombining populations (Melnikov et al., 2018), and horizontal gene transfer acts as a recurring compensating channel (Corradi, 2015). In some parasitic lineages reductive evolution reaches core biosynthetic capacity: lipid synthesis has been lost as a consequence of the parasitic lifestyle (Visser et al., 2010). Closely parallel reductive endpoints are documented in obligate bacterial symbionts, whose genomes are reduced toward genes for metabolic dependence and appear effectively irreversible (McCutcheon & Moran, 2012), illustrating the "dependence trap" in which short-term ecological gain is traded for long-term evolved dependence (Bennett & Moran, 2015). Obligate pathogens and symbionts also depend on host-derived lipids, further relaxing selection on their own metabolic modules (Nev et al., 2024). Comparisons across fungal pathogens indicate that pathogenicity-associated genes follow both divergent and convergent trajectories (Shang et al., 2016).

Two aspects of this material speak directly to the framework. First, no clearly documented case of an obligate parasitic group within the Dikarya reverting to a free-living state has been reported to date; the absence is compatible with the expectation that reversal becomes statistically constrained at very low integrity, but may equally reflect the detection limits and deep timescales that make rare reversals difficult to observe. Second, durable intermediate strategies are expected under the critical-zone logic: lineages within the constraint regime that have not yet crossed the zone.

Facultative parasitism and necrotrophy, now classified into several parasitism-saprotrophy strategies persisting over evolutionary timescales (Metz et al., 2025; Suzuki & Sasaki, 2019), are the primary instances; their stability may be held by antagonistic pleiotropy and the quasi-neutral plateau, in a near-neutral balance that directional selection does not readily resolve. The constraint regime is nonetheless evolutionarily active: clonal expansion and host-driven selection continue to shape population diversification in pathogenic lineages (Murante & Hogan, 2024).

Reversal from deep erosion, if it occurs, might travel by an exogenous route, with horizontal gene transfer compensating for module integrity from outside the module rather than by the stepwise restoration the framework treats as statistically inaccessible. The early-diverging chytrids, examined in case (9), provide the relevant fungal comparison: their lifestyle transitions appear to be accompanied by horizontal transfer and gene-family expansion rather than wholesale loss. The framework advances this only as a possible mechanism, not as a novel claim, and how far exogenous compensation can restore deeply eroded modules in the Dikarya remains an open question. The absence of documented reversals from obligate parasitism within the Dikarya, while compatible with the framework, does not by itself constitute strong evidence; absence of evidence and evidence of absence must be distinguished, especially given the practical difficulties of detecting rare reversal events on deep phylogenetic timescales.

(6) Lichenization, de-lichenization, and re-lichenization

When a lichenized lineage loses its photobiont and returns to a free-living state, the framework would predict a differential pattern of module retention. Genes whose functions were rendered redundant during the lichenized phase, such as pectinases if the photobiont cell wall lacks pectin, should show deep loss; genes whose functions

remained under selection, such as mycelial development genes, would be retained. Lignocellulose-degrading genes occupy an intermediate position: their retention might vary depending on the degree to which they retained functional relevance during the lichenized phase and the potential for future saprotrophic reversion.

This differential pattern appears to be supported to some extent. Most sequenced lichenized fungi have undergone genome reduction, with extensive loss of genes encoding plant cell-wall-degrading enzymes. Pectinase genes, for example, are nearly completely lost in multiple lineages, possibly related to the absence of pectin in the cell walls of symbiotic algae (Song et al., 2022). However, genes related to lignin and cellulose degradation are retained to a considerable extent in some lichenized lineages, with certain groups reportedly possessing numbers of carbohydrate-active enzyme genes comparable to those of saprotrophic species (Resl et al., 2022). Genes involved in mycelial architecture and development, in contrast, tend to be highly retained or even show lineage-specific innovations (Song et al., 2022). The numerical asymmetry between gain and loss is also documented: within pyrenocarpous ascomycetes, at least 12 lichenization events, 3 re-lichenization events, and at least 24 de-lichenization events have been inferred (Aptroot, 1998), and the same fungal species may be lichenized on one substratum and saprotrophic on another (Wedin et al., 2004).

The differential retention pattern is compatible with the module-specific degradation logic. Pectinase genes, rendered redundant during the lichenized phase, show deep loss; lignocellulose-degrading genes, potentially retaining functional relevance or a re-activatable basis for saprotrophic reversion, show intermediate retention; mycelial development genes, continuously under selection, show high retention. The causal association with the framework's specific mechanism, relaxed selection on bottleneck cores leading to downstream degradation, has not been directly tested in lichenized

lineages.

If de-lichenization involves module degradation, then re-lichenization might require either sufficient retention of the lichenization-related modules, or a lowered initiation threshold through low-specificity partner choice, or compensatory mechanisms such as gene family expansion, neofunctionalization, or horizontal gene transfer. The framework would predict that re-lichenization events should be more frequent than reversal from obligate parasitism, because the degree of module degradation associated with lichenization is typically less severe.

The re-acquisition of lichenization appears in the phylogenetic record with a certain frequency, including at least 3 inferred re-lichenization events within pyrenocarpous ascomycetes (Aptroot, 1998) and possible re-acquisition events following loss in other clades (Thiyagaraja et al., 2020). The threshold-lowering reading finds some support in several observations. Phylogenetic studies show that symbiont switching occurs repeatedly in lichen evolution, with fungi and algae generally not exhibiting strict co-speciation patterns (Piercey-Normore & DePriest, 2001; Chagnon et al., 2019); population genetic studies indicate that the two partners may disperse independently and re-establish relationships in new habitats (Romeike et al., 2002). More directly, experimental evolution has shown that a mutualism between a yeast and an alga can arise spontaneously under conditions requiring reciprocal exchange (Hom & Murray, 2014). The initiation of such symbioses may therefore depend more on ecological context than on the prior evolution of a dedicated molecular program. Under experimental conditions, lichen fungi can establish temporary associations with non-preferred algae (Guzow-Krzemińska & Stocker-Wörgötter, 2013), their partner-choice strategies at fine genetic scales may be broader than patterns observed at the species

level (Ossowska et al., 2024), and in anthropogenically disturbed habitats even non-customary algae may serve as alternative partners (Osyczka et al., 2021). The availability of partners is itself a historical variable: the earliest clear lichen fossil predates the Trentepohliales radiation (Taylor et al., 1997; Yuan et al., 2005), which may help explain why this algal group became an available low-cost pathway only in later lichenization evolution. Lichenization is ancient and has been inferred as ancestral for several major fungal lineages (Lutzoni et al., 2001). Phylogenomics has also begun to resolve the algal side of lichenization (Puginier et al., 2024).

A complementary perspective on the fungal side of re-lichenization concerns the symbiotic form itself. Goward (2009a, 2009b, 2010) has proposed that lichen thalli may have arisen repeatedly from pre-existing, loosely organized fungal-algal associations, termed paralichens, that functioned as nutrient-rich "germination beds" rather than as fully integrated symbiotic structures. In this view, the lichen thallus is not constructed de novo each time; it emerges as a "systems upgrade" from an already functional relational interface between partners. This perspective bears specifically on the fungal partner's capacity to re-enter a lichenized state after loss. If re-lichenization can proceed through paralichen-like intermediates, the initiation threshold may be lower than a full rebuilding of the ancestral lichenization program would imply. A fungal lineage that has lost some genetic components of the lichenized condition might still participate in a loosely organized association that, under suitable ecological conditions, subsequently gives rise to a fully organized thallus. This does not replace the genomic logic of module integrity; it identifies an additional, relational dimension, the availability and compatibility of partners at the interface, along which the threshold for re-lichenization can be lowered.

The frequency of re-lichenization, though modest, is consistent with the expectation

that transitions within a partially degraded state are more accessible than full reversal from deep degradation. The low-specificity partner-choice reading, with paralichen-like intermediates (Goward, 2010), suggests a lower initiation threshold than reconstruction of the full ancestral program would imply. Several mechanisms could provide alternative pathways: in lineages recently released from lichenization, related genes may not yet be fully degraded; gene family expansions, neofunctionalization, or horizontal gene transfer might substitute for key functions (Schmitt & Lumbsch, 2009); and at shorter timescales, transcriptomic reprogramming and epigenetic plasticity (Valim et al., 2025) could provide immediate regulatory response space.

Symbiont identity and symbiotic phenotype do not translate simply into lineage diversification in lichens (Nelsen et al., 2020); the ecological consequences of re-lichenization should not, therefore, be conflated with the molecular feasibility of the transition itself. Current research on the molecular mechanisms of lichenization loss and re-lichenization remains relatively preliminary, and the testing of these interpretations awaits future work. The case illustrates the framework's general stance: the constraining effect of module integrity would not operate in a vacuum, since external ecological conditions, intrinsic partner-selection flexibility, and multi-level genetic and epigenetic regulatory mechanisms might together shape the macro-patterns of complex trait reversibility. Diversification dynamics among lichen-forming fungi are heterogeneous (Kraichak et al., 2015a; Kraichak et al., 2015b).

(7) Horizontal gene transfer and module compensation

The framework would predict that the functional categories of horizontally acquired genes should differ systematically between integrity regimes: low-integrity lineages, whose core metabolic modules have been degraded, might acquire genes that compensate for lost core functions; high-integrity lineages, whose core modules remain

intact, might acquire genes that expand peripheral metabolic capacities. This prediction, stated as Expectation 4 in the main text, is the framework's most concrete interface with the HGT literature.

The survey evidence is broadly, though only indirectly, consistent with this expectation. Across 829 representative fungal genomes and more than 20,000 prokaryote-derived genes, parasitic fungi carry the highest numbers of acquisitions, and gasteroid (closed-fruitbody) lineages the highest among morphological states (Liu et al., 2025). Wood saprotrophs show an enrichment of acquisitions in transporters, signaling components, and protein-folding factors, which are auxiliary categories rather than core free-living metabolism. The case-level evidence is consistent with the compensatory reading in both directions: in chytrids, acquired genes appear to accompany lifestyle transitions and expand metabolic capacity without replacing eroded cores (Chrismas et al., 2025); microsporidia combine extreme gene loss with recurring acquisition (Corradi, 2015); and within a single secondary-metabolite cluster surveyed across 585 genomes, horizontal transfer and gene loss seem to act together to shape retention (Reynolds et al., 2017).

Parasitic and gasteroid lineages, predicted to occupy the low-integrity regime, show the highest HGT counts; wood saprotrophs, predicted to occupy the high-integrity regime, are enriched in auxiliary categories rather than core metabolism. Both observations are consistent with the functional-category expectation, and both are correlative: acquired genes are frequently lost again (Richards et al., 2011), transfer inferences depend on methods and taxon sampling (Szöllősi et al., 2015), adaptive-transfer identification carries well-known caveats (Soanes & Richards, 2014), and acquisition counts respond to many factors of which module integrity is one (Liu et al., 2025).

A rigorous test of Expectation 4 would require a targeted design: lineages with

independently measured integrity scores, their HGT-acquired genes classified by the module compensated, and the enrichment contrast across integrity regimes (Section V). The genomic resources for such a test now exist; the test itself remains to be performed.

(8) Fungal viruses, endosymbionts, and the extended fungal entity

The networked architecture of mycelia, a core structural premise of the framework, implies that entities transmitted through hyphal fusion might be subject to distinctive evolutionary dynamics. Most mycoviruses lack extracellular stages, depend on hyphal fusion for transmission, and often exert little phenotypic effect (Ghabrial et al., 2015; Nuss, 2005). Over 80 formally recognized species spanning ten families are known, with dsRNA viruses dominant and ssRNA viruses approaching one third (Pearson et al., 2009). The widespread coexistence of low virulence with fungal hosts is consistent with the expectation that networked ecological strategies might reduce selection pressure favoring the evolution of high virulence. Viruses can also modify module output without changing the fungal genome. In the classic three-way system, a virus infecting a fungal endophyte confers thermal tolerance on the plant host, and curing the fungus of the virus abolishes the trait (Márquez et al., 2007). Hypovirulence-associated mycoviruses illustrate the same principle in the reverse direction (Azhar et al., 2019).

The viral case is best read within a wider phenomenon. Fungal cells harbour diverse heritable endosymbionts, including viruses, bacteria, and other cytoplasmic elements, and these may together constitute a composite evolutionary unit rather than a set of independent passengers. A loose parallel is available from the amoebae, which have been described as evolutionary melting pots in which phagocytic hosts acquire, mix,

and retain genetic material from diverse sources, generating chimeric genomes at unusually high rates (Boyer et al., 2009). Fungi may occupy an analogous position in terrestrial systems, not through phagocytosis but through the syncytial mycelium, which permits the coexistence and recombination of cytoplasmic elements across hyphal compartments and through anastomosis. How far this parallel extends remains open, since the mechanisms of cytoplasmic inheritance and exclusion in fungi differ substantially from amoeboid phagocytosis. It nonetheless suggests that the fungal individual may be better treated as a consortium than as a single genome.

Several consequences follow for evolutionary dynamics. Viruses can act as vectors of horizontal gene transfer, and although the evidence remains indirect, this would provide a channel through which genetic material moves between fungal lineages without sexual reproduction (Ghabrial et al., 2015). Viral transmission through the mycelial network need not require high within-host replication or severe tissue damage, since a network can potentially compartmentalise infected regions while maintaining overall fitness, and this may favour temperate or persistent strategies over lytic ones. The relationship is also likely to be coevolutionary rather than unidirectional. Fungal hosts may evolve tolerance or exclusion mechanisms, and viruses may evolve counter-strategies, with the outcome shaped by the same networked architecture that structures fungal ecology. Beyond direct effects on virulence, endosymbionts may influence fungal phenotypes through indirect routes, including altered metabolic flux, stress tolerance, and developmental timing, and such effects could in principle feed back on the morphological and ecological variation that the framework addresses.

These observations are offered as extended inferences rather than tested claims. The role of viruses as horizontal transfer vectors in fungi remains speculative, the extent to

which endosymbiont communities function as integrated evolutionary units is unresolved, and the amoeba analogy is a heuristic rather than a demonstrated mechanism. What the material suggests is a broader point. The fungal evolutionary trajectory may be shaped not only by the fungal genome and its developmental architecture, but also by the cytoplasmic and intracellular associates that travel with it. Whether these associates constitute a significant and systematic influence on fungal macroevolutionary patterns, rather than a source of idiosyncratic variation, is a question the framework does not settle and that future work could address.

(9) Chytrids: the shallow end of the erosion gradient

By the framework's logic, the depth of achievable module erosion should scale with architectural depth (Section IV(3)). Lineages whose developmental systems are simple, with few modules and bottleneck cores commanding modest target sets, should be able to enter parasitic states with only shallow erosion, and should therefore retain a measurable capacity to return. Where parasitic adaptation proceeds by addition rather than subtraction, ancestral free-living modules would remain largely intact, and reversal would remain statistically accessible rather than closed.

The chytrids, as the earliest-diverging fungal lineage, appear to occupy exactly this regime. Phylogenomic analysis of phytoplankton-infecting chytrids, combining transcriptomic and single-cell genomic data, has inferred multiple independent lifestyle transitions between parasitism and saprotrophy across the phylum, in both directions; the chytrid last common ancestor was possibly itself a parasite of phytoplankton (Thomé et al., 2024). The molecular correlates of this flexibility are correspondingly additive. Comparative genomics of pathogenic Batrachochytrium species against saprotrophic relatives identified remarkable expansions of protease and cell-wall gene families, rather than the wholesale gene losses characteristic of obligate parasites in

higher fungi (Farrer et al., 2017); pan-genomic analysis of Batrachochytrium dendrobatidis documents substantial copy-number variation in pathogenic gene families, suggesting population-specific and non-deterministic responses to parasitism (Yacoub & Stajich, 2025). Most directly, Kelly et al. (2021) showed that isolates of the amphibian pathogen Batrachochytrium salamandrivorans sustain facultative saprotrophic life cycles when hosts are absent, a direct observation that the free-living program remains functional within a pathogenic lineage. At the saprotrophic pole, the chitin-utilizing chytrid Rhizoclosmatium globosum illustrates how free-living capacity can be built additively, through chemotaxis toward chitin monomers, a diversified GH18 chitinase secretome, and bacteria-like chitin-binding domains acquired by horizontal gene transfer (Chrismas et al., 2025; Ciach et al., 2024).

The material relates to the framework at three points. The bidirectional transition pattern makes the predicted high-reversibility regime visible: where erosion is shallow, both directions remain open. The additive character of chytrid parasitism, expansion and acquisition rather than loss, matches the expectation that specialization need not move a lineage toward the critical zone. And the facultative saprotrophy of Batrachochytrium salamandrivorans comes close to a direct observation of the predicted intermediate state, a lineage still within the basin from which return remains possible. The chytrid contrast is the framework's outgroup test: if the depth-of-erosion gradient (Section IV(3)) is real, reversal frequencies should be highest among developmentally simple lineages and decline with architectural depth (Expectation 6).

The chytrid case rests on a single phylum whose molecular characterization remains incomplete, and the inference of bidirectional transitions depends on ancestral state reconstructions whose robustness has not been examined as extensively as in the Agaricomycetes literature. The apparent kingdom-scale gradient may also partly reflect

detection and timescale limits rather than mechanism alone. The contrast therefore remains a promising pattern for further study, not a settled demonstration.

Taken together, these cases are best read not as independent anecdotes but as candidate manifestations of a single process operating at different positions along the module-integrity spectrum. Their evidential weight varies, from well-quantified macro-patterns to inferences that still await genomic resources. Rather than settling these cases, the framework helps make their shared regulatory logic explicit and specifies what evidence would confirm or challenge each reading.

Supplementary Table 1 | Summary of Supplementary Box 2 case studies

Case	Framework expectation	Evidence status	Principal caveat
(1) Fruiting bodies and hymenophores	Peripheral-node transitions appear frequently reversible; bottleneck-core transitions show strong asymmetry; a spectrum of directional constraint	Graded pattern observed: open hymenophore types transition bidirectionally; some directionality exists but neither direction fully closed. Compatible with	Regulatory-hierarchy interpretation remains hypothesis; specific genes and architectures underlying transitions not mapped.

		framework.	
(2) Gasteroidization as developmental truncation	Truncation at a bottleneck core predicts frequent forward transitions, near-zero reverse, simple genetic basis, ecological permissiveness, and a temporal gap before lock-in.	Strong support: 123 independent origins, reverse unlikely; *Lentinus tigrinus* single-locus case; coexisting morphs support temporal gap. Extension to smooth hymenophores plausible.	Truncation model not tested at specific bottleneck-core level; smooth hymenophore developmental genetics insufficient.
(3) Yeast-hypha dimorphism	Dimorphic fungi provide tractable test: bottleneck-core loss → directional constraint; peripheral changes → reversibility.	Natural replicates and molecular tools exist (*S. pombe* vs. *S. japonicus*, Mucorales, *C. albicans SSN3*); not yet direct evidence for framework.	Current evidence insufficient for firm conclusions; systematic cross-lineage comparisons not yet performed.
(4) Mycorrhizal	Reversibility rank:	Genomic patterns	Reversal

gradient	ECM intermediate, ericoid/orchid higher, AM deepest degradation; module-specific integrity loss.	broadly consistent: ECM reduced PCWDEs but retained some; ericoid/orchid high retention; AM deep degradation; *Amanita* shows graded erosion.	inferences depend on phylogenetic model assumptions; signals are preliminary, not settled measurements.
(5) Parasitic strategies and the critical zone	Obligate parasitism = extreme low-integrity endpoint; facultative/necrotrophic states occupy the constraint regime but have not crossed the critical zone.	Extensive module erosion documented in obligate parasites; no clear reversal in Dikarya; intermediate strategies common and stable.	Absence of reversal is not strong evidence; detection limits and deep timescales confound interpretation.
(6) Lichenization, de-lichenization, re-lichenization	De-lichenization: differential retention (pectinases lost, lignocellulose	Differential retention supported; re-lichenization	Molecular mechanisms preliminary; causal link to

	intermediate, mycelial retained). Re-lichenization more frequent than reversal from obligate parasitism.	events inferred; low-specificity partner choice supported.	bottleneck-core relaxation not tested.
(7) HGT and module compensation	Low-integrity lineages acquire genes compensating eroded cores; high-integrity lineages expand peripheral capacities.	Large-scale survey broadly consistent: parasitic/gasteroid highest HGT; wood saprotrophs auxiliary categories; case-level support.	Correlative and indirect; direct test requires classifying acquisitions by module and comparing integrity regimes.
(8) Fungal viruses, endosymbionts, and the extended fungal entity	Networked mycelia permit hyphal-fusion transmission of cytoplasmic associates, which may form a composite evolutionary unit; viruses may act as HGT vectors.	Mycoviruses mostly low virulence and persistent; three-way symbiosis shows virus-mediated trait transfer; diverse heritable	HGT vector role speculative; extent of endosymbiont integration unresolved; amoeba parallel is analogy, not homology

		endosymbionts documented; amoeba “melting pot” analogy heuristic	
(9) Chytrids: shallow end of erosion gradient	Simple architecture → shallow erosion; additive specialization retains free-living modules; bidirectional transitions expected.	Chytrids show bidirectional parasitism-saprotrophy; additive expansions; facultative saprotrophy observed.	Single phylum; incomplete molecular characterization; ancestral-state inference robustness not thoroughly examined.

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