









An integrative paleolimnological approach for studying evolutionary processes

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Abstract

The field of paleolimnology has made tremendous progress in reconstructing past biotic and abiotic environmental conditions of aquatic ecosystems based on sediment records. This, together with the rapid development of molecular technologies, provides new opportunities for studying evolutionary processes affecting lacustrine communities over multicentennial to millennial time scales. From an evolutionary perspective, such analyses provide important insights into the chronology of past environmental conditions, the dynamics of phenotypic evolution, and species diversification. Here, we review recent advances in paleolimnological, paleogenetic and molecular approaches, and highlight how their integrative use can help us better understand the ecological and evolutionary responses of species and communities to environmental change.

Recent advances in paleolimnology

Recent developments in multi-proxy paleolimnological approaches and chronological methods have substantially advanced our ability to make robust inferences about past environments [1–3], including changes in regional climate, water chemistry, and community composition (Box 1) [4–6]. For example, advances in both isotope geochemistry and spectroscopy analyses of lake sediments have improved the characterisation of past thermal and chemical environments of lakes, as well as their productivity and catchment connectivity [7,8]. Additionally, the reconstruction of past community composition and dynamics has been facilitated by sequencing (i) environmental DNA from bulk sediment that lacks discernible subfossils (i.e. sedimentary ancient DNA, **sedaDNA**, see Glossary) [9], and (ii) organismal remains preserved in the sediment (i.e. ancient DNA, **aDNA**) (Box 2) [10,11], opening new insights into the genetic composition of past populations.

Such advances in the field of **paleolimnology** provide new opportunities for integrative studies of how past biotic and abiotic environmental conditions can influence key ecological and evolutionary processes (Table 1). For example, through the recovery of **microremains** or dormant stages of cladocerans (i.e. **resurrection ecology**), previous studies have revealed shifts in predator-mediated selection [12], adaptation to environmental pollutants [5], and co-evolution with parasites [13]. Recovering fish **macroremains** (e.g. teeth) from sediment cores can help track morphological evolution of ecologically relevant traits [14] and past patterns of fish community composition [15]. Applying these methods within a broader paleolimnological approach could help us understand both microevolutionary dynamics of populations (e.g. rates and causes of adaptive evolution) and macroevolutionary patterns in limnic environments (e.g. **adaptive radiations**). In this review, we highlight how an integrative paleolimnological approach can improve our understanding about the past environmental conditions and population dynamic processes of evolution, as well the underlying mechanisms of natural selection and adaptive radiation (Figure 1).

Reconstructing past environmental conditions relevant to evolution

Long-term records established by lacustrine sediments are useful for understanding the ecological context of how species and populations evolve in response to environmental changes in natural ecosystems. Paleolimnological records can elucidate the nature and timescale of both endogenous (i.e. internal) and exogenous (i.e. external) drivers of environmental change that can influence evolution. For instance, changes in lake morphometry (e.g. depth, surface area, shoreline extent) can be inferred from reconstructions of lake level changes and hydroclimatic variability (e.g. using methods described in Box 1), ideally in combination with detailed basin,

topographical, and sedimentological analysis (i.e. sedimentation rates). In Lake Ohrid, an ancient Balkan lake, reconstructing the dynamics of lake expansion and deepening, helped explain the timing of diversification of diatoms [16] and macroinvertebrate clades [17], possibly due to increased ecological opportunity. Similarly, in East African Rift Valley Lakes, changes in water level have coincided with the spectacular dynamics of cichlid fish diversification and extinction [18,19].

Lake sediments can also reveal ecological and geomorphological changes that have occurred in the surrounding landscape, such as fire-induced shifts from forest to grassland vegetation [20] or changes in the influx of nutrients and organic matter [21]. Such exogenous change can influence the ecological context of evolution. For example, lake water clarity is an important determinant of visually mediated behaviours in aquatic animals (e.g. resource and mate acquisition). Reconstructing long-term patterns of water clarity, for example from indicators of algal productivity and turbidity, could provide an important historical perspective for understanding how visual mating cues can affect the reproductive isolation and coexistence of species [22]. In addition, documenting the timing of past changes along environmental gradients, such as lake oxygenation, resource availability (e.g. pelagic/benthic), and habitat structure (e.g. macrophyte beds), is relevant for understanding both the origin of lacustrine species and when these conditions change the underlying dynamics of species extinction [23]. More generally, reconstructing past environments is an important starting point for understanding the ecological context of adaptive evolution, by providing insights into the dynamics of populations and the causes of natural selection.

Reconstructing population dynamic processes of evolution

Paleolimnological studies have made substantial contributions to our understanding of past population dynamics [24], contemporary species distribution patterns [25], biological invasions [26], and gene flow among populations [27]. Here, we propose that the use of paleolimnological data can help further reconstruct the past dynamics of populations, and their connectivity. Existing genomic methods for estimating the demographic history from contemporary sequence data typically suffer from considerable levels of uncertainty of population dynamics through time [28]. Paleolimnological data could help validate whether population size fluctuations inferred from genomic data are consistent with historical abundance records obtained from macro- or microremains, or if they are plausible given reconstructed environmental conditions. Similarly, we propose that co-occurrence patterns derived from sediment cores (e.g. using methods in Box 2) can help interpret the timing of past gene flow among and within lineages, which is typically inferred from genomic analyses of contemporary lineages.

Morphological remains in sediments can provide insights into the timing of lineage colonization in lakes, and in some cases contribute genetic data from ancient lineages (i.e. a **paleogenomic** perspective) [29]. For example, patterns of genetic similarity among lineages recovered from sediments (e.g. DNA extracted from diapausing eggs and morphological remains) have been used to investigate genetic changes within a lineage over time, and distinguish between gene flow from secondary contact and selection on standing genetic variation [30,31]. Similar studies conducted on multiple lakes can help uncover the timing of lineage co-occurrence within and among populations, providing insights into the history of colonization and population connectivity [32]. An explicit integration of paleolimnological proxies with paleogenomic approaches can reveal further insight into past evolutionary dynamics. Dean et al. used compositional analyses of sediment elements and diatoms alongside genomic data from contemporary populations to estimate the history of colonization and spatial isolation between lineages of threespine stickleback (*Gasterosteus aculeatus*) in coastal Scottish lagoons [33]. Extending this approach, a study in Norwegian lakes used sediment analysis together with contemporary stickleback genomes and two partial genomes extracted from stickleback macroremains collected near the

period of lake formation (i.e. the founding population) to study the influences of demography and natural selection spanning ~13,000 years of evolutionary history for these populations [11].

Improving our inference about past selective environments and rates of evolution

Identifying the environmental processes influencing evolution by natural selection is a pervasive challenge in evolutionary ecology research [34]. Often, **selective environments** are influenced by multiple interacting biotic and abiotic factors and the link between organismal phenotype and fitness is both complex and environmentally contingent [35]. Integrating paleolimnological evidence with geological records of phenotypic variation allows us to examine how changing ecological conditions drive morphological variation in key taxa and test hypotheses about past selective environments [5,14]. The continuous archives of phenotypic data preserved in lake sediments spanning from annual to millennial timescales provide unique and complementary insights into the temporal dynamics of phenotypic evolution. This connects with a fundamental goal in evolutionary research, which is to understand the pace of phenotypic evolution over a range of timescales [36,37].

The nature and pace of evolutionary responses can be studied directly from organisms that are resurrected from lake sediments. Dormant stages of some species (e.g. diapausing eggs, spores, resting cells) that are decades or centuries old have the potential to be reared and studied [38]. Such approaches have been particularly useful for studying adaptation to human-mediated environmental changes that have occurred over the past 200 years, including acidification, soil erosion, or water quality change such as pH or nutrients. Considerable progress has been made by resurrecting organisms from lake sediments [38], and experimentally testing putative agents of selection, such as pesticide use [39], predation regimes [12], and nutrient abundance [31]. For example, Hairston *et al.* hatched *Daphnia* resting eggs from Lake Constance sediments and used laboratory common garden experiments to show evolution of increased tolerance to toxic cyanobacteria [40].

If organisms cannot be resurrected, then evolution can be inferred by studying changes in both phenotypes and allele frequencies, provided the remains are well preserved for the relevant analyses. For example, body size variability in cladoceran populations can be compared to morphological analyses of remains preserved in lake sediments along past environmental gradients [41]. Using morphological analysis of macroremains (e.g. carapaces, headshields, and post-abdominal claws) recovered from sediment cores, Korosi *et al.* found rapid reductions in cladoceran body size from pre- to post-industrial conditions. These phenotypic responses likely resulted from a combination of warming, increased acidification, and increased predation [42]. Such phenotype-by-environment correlations over time can complement both comparative and experimental studies of selection in contemporary populations. For example, the integrative approach used by Frisch *et al.* reconstructed changes in environmental conditions, allele frequencies (over 1600 years), and phenotypes (i.e. from common garden experiments using resurrected individuals up to 700 years old), in order to understand evolutionary responses of *Daphnia* to cultural eutrophication [31]. More generally, we think that a greater emphasis on evolutionary analyses of remains in lake sediments can complement ongoing efforts to understand evolutionary rates, and the putative drivers of adaptive evolution.

Understanding the drivers of adaptive radiations

Despite extensive research on adaptive radiations, fundamental questions regarding their timing and dynamics remain unanswered [43,44]. Paleolimnological approaches could help characterize the environmental circumstances at the onset, and over the course of adaptive radiation. For example, as mentioned above, sediment records documenting lake-level fluctuations have provided an important environmental context for interpreting several lacustrine radiations [17,18]. In another example, catastrophic volcanic events, as inferred from paleolimnological

evidence [45], could explain why the cichlid fish community of Lake Kivu is astonishingly species poor compared to other East African Rift lakes [46].

Paleolimnological approaches can also be used to quantify patterns of community assembly of a radiating clade and co-occurring species (e.g. analysing morphological remains, sedDNA, and aDNA). A classical hypothesis in adaptive radiation research is that radiations occur when a colonizing lineage finds itself freed from competitors in a novel adaptive zone [47]. Fish teeth extracted from sediment cores reaching back to the very origins of Lake Victoria revealed that potential competitors of haplochromine cichlids were already present in the earliest stages of lake refilling ~15,000 years ago, alongside haplochromines, providing a community context to the early onset of radiation [15].

One of the outstanding and challenging questions in adaptive radiation research is what drives variation in rates of diversification among and within lineages. A paleolimnological perspective is particularly powerful because it can include the dynamics of extinct lineages for which there are no contemporary records. For example, a previous study has linked the rate of trait diversification within a gastropod radiation with long-term changes in climate and lake-level fluctuations [48]. A similar approach applied to other taxa that preserve morphological remains in lake sediments could also be used to track the progression of adaptive radiations [14]. In addition to morphological evidence from macroremains, recovering genetic material from sediments and fossils (Box 2) might provide an additional source of inference about the underlying drivers of variation in diversification rates among and within lineages. We are cautiously optimistic that the development of aDNA methods will considerably advance our understanding of adaptive radiations in lacustrine systems.

Concluding Remarks and future perspectives

Despite the wide advances on paleolimnological approaches in recent years, there are several challenges related to the interpretation of paleolimnological data. For instance, physical disturbance (e.g. bioturbation) can lead to reworking of the sediment layer and a poor temporal resolution of the sediment, which in turn will affect the interpretation of rates of evolutionary and ecological change. Additionally, sediments with different preservation status will influence the reliability and interpretation of different proxies, including those biological proxies that are particularly relevant for evolutionary studies (see limitations in Box 1: organism remains, and Box 2: molecular analyses).

A growing number of studies have used integrative and multi-proxy approaches from both paleolimnology and evolutionary biology to address novel questions at the interface of these two disciplines [49,50]. As new environmental, phenotypic, and molecular technologies emerge (Box 1 and 2), we expect this trend to continue and intensify. We anticipate that paleolimnological records will be used more frequently to understand evolution in the context of past selective environments, the timing of species colonisation, and the process of adaptive radiation (Figure 1). Gleaning information from paleoenvironmental and evolutionary reconstructions will eventually help us forecast responses of species and communities to changing environments (see Outstanding Questions). It would be particularly exciting if integrative approaches combining genomics, morphology, and paleoenvironmental proxies could shed light on feedbacks between environmental and evolutionary dynamics. We are hopeful that such paleo-evolutionary approaches will improve our understanding about the evolutionary histories of species, and better explain their adaptive capacity in the face of continued changes to the environment [51].

Glossary:

Adaptive radiation: the evolution of ecological and phenotypic diversity within a rapidly diversifying lineage.

Ancient DNA (aDNA): DNA recovered from subfossil and fossilized remains of organisms (e.g. bones and diapausing eggs), characterised by short molecules and post-mortem chemical damage. The term is also used for samples that are only years or decades old if the DNA is degraded.

Macro- and micro-remains: parts of organisms that accumulate in aquatic sediments, being partially or entirely preserved. Macroremains typically refers to structures visible by eye, whereas microremains are only visible with microscopy.

Paleogenomics: An emerging field of study focusing on the methodologies and analyses of aDNA.

Paleolimnology: Multidisciplinary field studying the long-term development of inland water ecosystems, usually focusing on the physical, chemical, and biological properties of aquatic sediment through time.

Resurrection Ecology: the study of traits and responses of past populations to environmental variability by hatching dormant propagules of species.

Sedimentary ancient DNA (sedaDNA): DNA that can be extracted from bulk sediment, residing in, or being adsorbed to organic or inorganic particles, especially clay minerals.

Selective environment: Environmental conditions causing differential fitness among individuals in a population.

Table 1. Selected studies of evidence of evolution in taxa integrated with paleolimnology analyses. Acronyms: LOI, loss-on-ignition analysis.

Ecological or evolutionary phenomenon	Temporal scope (years BP)	Target organism(s)	Change in environmental conditions	Change in target organism(s)	Integrative paleolimnological approach	Citation
Demographic change	~300	Pacific salmon (<i>Oncorhynchus nerka</i>)	Increase of temperature Increase of artificial fertilizers Increase in commercial fishing	Decrease in population size	Isotopic analysis of $\delta^{15}\text{N}$ Diatoms and cladoceran community analysis	[52]
Demographic change	~200	<i>Daphnia</i> spp. <i>Bosmina</i> spp.	Decrease in Calcium concentration Acidification (pH<5.7)	Decrease in relative abundance of large-bodied cladocerans	Diatoms-based pH reconstruction Past variability of Ca concentrations	[53]
Demographic change	~18,000	Chironomidae	Increase of temperature	Decrease in population size of cold-adapted taxa	Chironomid-temperature transfer functions Pollen analysis Geochemical analysis	[4]
Timing of colonization	~10,000	Whitefish (<i>Coregonus lavaretus</i> L.)	Waterfall emerged (isostatic land uplift)	Morphologies and life-history changes.	aDNA Radiocarbon dating	[54]
Timing of colonization, Adaptation	~12,000	Stickleback (<i>Gasterosteus aculeatus</i>)	Lake insolation after LGM (glacial isostatic rebound)	Changes in allele frequency	aDNA Radiocarbon dating	[11]
Phenotypic evolution	~200	<i>Bosmina</i> spp.	Acidification (pH<5) Contamination of heavy metals	Body size variability Changes in antennule length	Taxonomic composition Diatoms-based pH reconstruction Sedimentary chlorophyll-a Sedimentary metal analysis	[5]
Phenotypic evolution	25,000	<i>Oreochromis hunteri</i>	Changes in water lake level	Changes in oral dentition	Taxonomic composition Radiocarbon dating	[14]
Phenotypic evolution	~100	Diatoms spp.	Increase of temperature	Decrease in mean size	Taxonomic composition ^{210}Pb and ^{137}Cs dating	[55]
Adaptation	~1,600	<i>Daphnia pulex</i>	Change in N:P ratio Increase of artificial fertilizers	Change in allele frequency linked with variability in P concentration	aDNA (dormant eggs) Total organic and inorganic matter (LOI) Sedimentary ortho-P analysis	[31]

Coevolution	~40	<i>Daphnia magna</i>	Parasite infection	"Red Queen" dynamics	Resurrection of <i>Daphnia magna</i> and <i>Pasteuria ramosa</i> resting eggs	[13]
Hybridization	~80	<i>Daphnia galeata</i> , <i>D. cucullata</i> and <i>D. longispina</i>	Colonization of new species (<i>D. galeata</i>)	Hybridization between <i>D. galeata</i> x <i>D. longispina</i> and <i>D. galeata</i> x <i>D. cucullata</i>	aDNA of dormant eggs and sediment	[32]
Hybridization	~12,000	<i>Bosmina</i> spp.	Colonization of new species (<i>Bosmina longispina</i>)	Change in appendage shape resulting from introgressive hybridization	Macroremains from <i>Bosmina</i>	[56]
Adaptive radiation	~11 My	Gastropod <i>Melanopsis</i>	Changes in lake level and climate.	Changes in shell geometry and reproductive isolation	Isotopes and geophysical analysis Fossil records	[48]
Adaptive radiation	~400,000	<i>Biwamelania</i> spp.	Lake expansion, lake level variability, novel habitats	Change in radula morphology and Karyotype variation (reproductive isolation)	Isotopes and geophysical analysis Fossil records	[6]
Adaptive radiation	~ 15,000	Haplochromine and oreochromine, cichlids, cyprinidae	Lake level variability	Radiation of Haplochromine cichlids	Taxonomic composition, subfossils Radiocarbon dating	[15]

Box 1. Classic Environmental proxies in paleolimnology

Paleolimnological research makes use of well-established biotic and abiotic proxies to reconstruct both terrestrial and aquatic environmental changes through time.

Basin and chronological analysis

Characterising basin geomorphology of lakes together with the chronology analysis of sediments are fundamental for both generating and interpreting paleoecological data [3]. Changes in lake basin morphometry (e.g. size and depth) have been shown to be the result of climatological conditions, tectonic processes and changes in sedimentation rate [57]. Long-term accumulation of sediments (i.e. geoarchives) are useful for reconstructing past hydrological connectivity among aquatic ecosystems [58]. In order to estimate sediment age, radiocarbon dating (^{14}C) is the most commonly used technique for carbonaceous materials over the past ~55,000 years [59] while more recent sediments (past ~200 years) are frequently done by using gamma spectroscopy (^{210}Pb and ^{137}Cs) [60]. Additional geochronological methods (i.e. sediment layers' components and structure) include varve counting, tephrochronology, and stratigraphic correlations [61].

Physical, chemical, and biological proxies:

Non-destructive analytical techniques (e.g. computerised tomography (CT) scans, visible-near-infrared (VNIR) spectroscopy and micro X-ray fluorescence (μXRF) scans) are commonly used to assess sediment stratigraphies, density, and elemental composition [3,62]. Following core splitting, additional geochemical analyses include measurements of organic matter content, biogenic silica, bulk isotope analysis (e.g. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$), and contaminants (e.g. metals) from past anthropogenic influences [63].

In addition to physical and chemical proxies, remains of aquatic and terrestrial organisms preserved in lake sediments, such as subfossil remains and biomarkers, allow the reconstruction of past changes in community assemblages. Researchers use known information about the ecology, distribution, and specific tolerances of key taxa (e.g. diatoms) to make inferences about past habitats, environments, and climate [2,64]. Plant macroremains, pollen, and charcoal provide key insights into forest and vegetation dynamics in the surrounding landscape [20], invertebrate macroremains can reveal changes in temperature, oxygenation, lake productivity, and anthropogenic impacts [65–67] and chemical biomarkers (i.e. lipids, pigments, or proteins) [68] can be used to reconstruct past populations dynamics in lakes (e.g. communities of green algae and cyanobacteria) [69].

Limitations and considerations

The temporal resolution of sediment cores and paleoenvironmental data is determined by environmental characteristics, stratigraphic quality, sediment deposition rates, and sampling decisions made by researchers [70]. Whereas physical and chemical proxies are influenced by the taphonomy and the reliability of sediment deposition. Proxies contained in older and deeper sediments are often subject to greater degradation, making it more difficult to interpret changes in both geochemistry composition and micro and macro-remains of organisms. Combining multiple paleolimnological techniques and collecting a large number of sediments can help improve the accuracy of the chronology and the reliability of the information extracted from sediment cores [71].

Box 2. Emerging molecular technologies

The rapid development of high-throughput sequencing technologies (HTS) provides new opportunities to study past evolutionary and ecological dynamics using DNA or proteins extracted from past sediment.

Genetic material is preserved in the sediment in different forms, including macrofossils, pollen grains, resting eggs, or DNA adsorbed to inorganic or organic matter [9,72]. DNA recovered from ancient samples (aDNA) can be used to track changes in species composition, genetic population structure, genetic variation, or gene flow, and reveal genetic relationships between ancient and modern samples [73]. While the extraction and analysis of aDNA has been developed into a powerful approach,

limitations remain. aDNA is usually only present in very small quantities, highly degraded, with molecules being short and chemically altered, and is admixed with DNA of non-target organisms such as bacteria and fungi. Thus, dedicated laboratories are required to minimize further contamination, and data analysis is complicated by post-mortem damage and short read lengths. These limitations are common to ancient DNA studies, but can be exacerbated in investigations of sediment archives, where organismal remains, such as fish, zooplankton or zoobenthos, are often minuscule. The water-logged environment might also facilitate the introduction of exogenous DNA into the sample. An additional limitation, at least currently, is the scarcity of genomic reference data from aquatic taxa other than vertebrates, potentially hampering comprehensive analysis of aDNA data.

In addition to DNA, ancient proteins from macrofossils can also be informative for a range of questions. Proteins can be extracted and used for fingerprinting by mass spectrometry, revealing the taxonomic identity of samples [74]. With more effort, the amino acid sequence of the most abundant proteins or even a large part of the proteome can be determined, enabling phylogenetic reconstructions [e.g. 75] or inferring differences in expression patterns [e.g. 76] not readily revealed by aDNA analysis. Ancient proteins often outlast aDNA, owing to greater chemical stability, and allow for much older samples to be successfully analysed. So far, this approach is not widely used on sediment records and here similar limitations might apply for aDNA analysis, namely, the small size of individual samples and possibly increased contamination with exogenous protein. Current research is aiming to increase the robustness and efficiency of both paleogenetic and paleoproteomic methods and establish reporting standards [77,78].

Figure 1. Illustration of a sediment core. Potential information acquired from a hypothetical sediment core integrated with relevant environmental scenarios (left panels) and examples of information extracted from a sediment core (right panels). Sediment records are informative for evolutionary studies by quantifying past environments, reconstructing past demography, inferring natural selection and the pace of evolution, and comparing these with changing environments (e.g., rapid evolution of pollution tolerance by zooplankton). Paleolimnological records can also help to reconstruct adaptive radiations and compare these with past environments.

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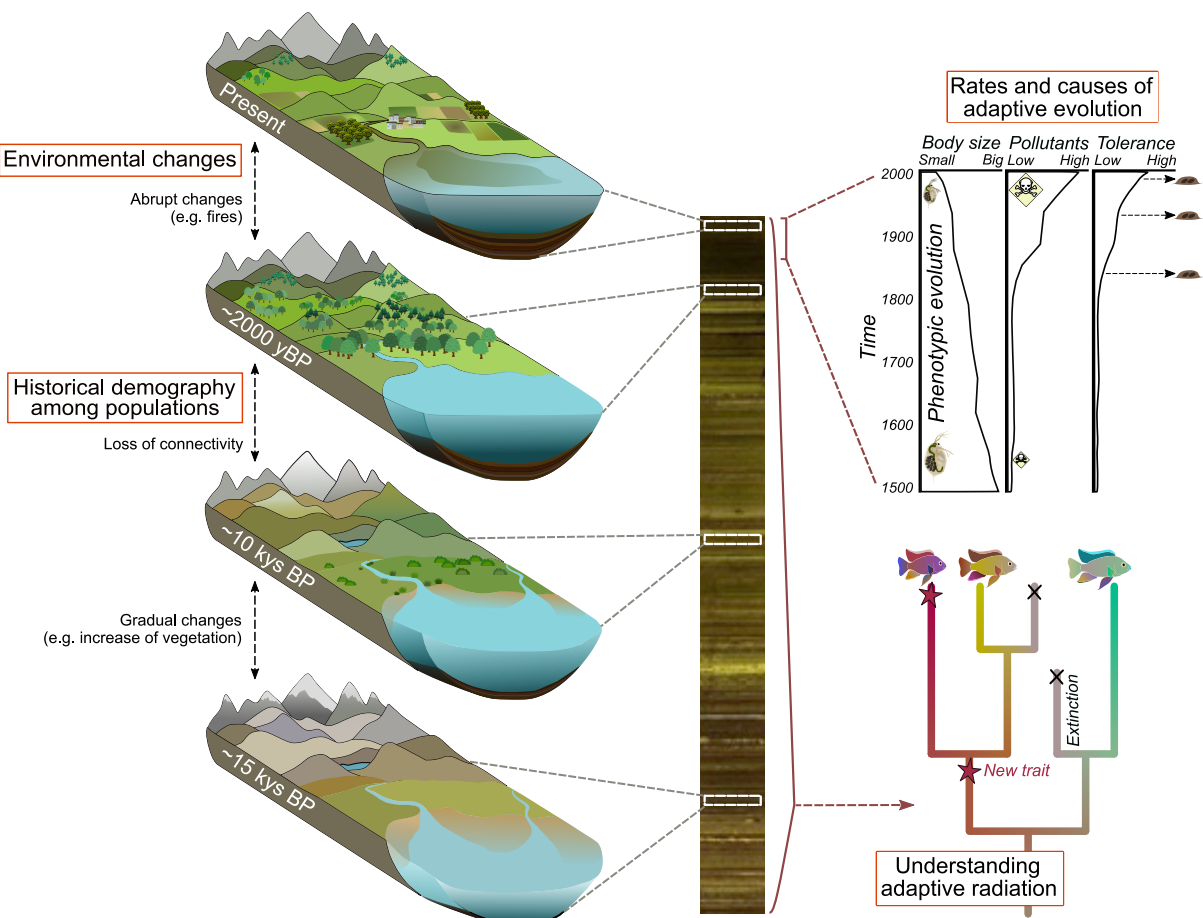
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Figure



An integrative paleolimnological approach for studying evolutionary processes

Maria Cuenca-Cambronero ^{1,2,3} , Colin J. Courtney-Mustaphi ^{4,5} , Ryan Greenway ¹ , Oliver Heiri ⁴ , Cameron M. Hudson ¹, Leighton King ^{1,2}, Kimberley D. Lemmen ⁶ , Marvin Moosmann ^{1,2}, Moritz Muschick ^{1,2} , Nare Ngoepe ^{1,2}, Ole Seehausen ^{1,2} , Blake Matthews ^{1,2} .

Outstanding questions:

Can an integrative paleolimnological approach provide new insights into evolutionary dynamics over a range of time scales, connecting micro- to macroevolutionary change?









Can paleolimnological approaches yield novel eco-evolutionary time series of populations and communities?

Can paleolimnological data validate demographic inference from genomic data, such as historical population sizes and gene flow among populations and species?

How robust are emerging proxies and novel technologies, and how informative are they over a range of environmental settings?

Can an integrative and retrospective paleolimnology approach help us understand the drivers of contemporary biodiversity change?

An integrative paleolimnological approach for studying evolutionary processes

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Highlights:

Paleolimnological data can help reconstruct past environmental conditions and their changes over time in aquatic ecosystems, providing important information for inferring past ecological conditions, trends in community assembly through time, and rates and directions of evolution.

New approaches to analysing ancient sedimentary DNA, RNA or proteins, lipid biomarkers, and stable isotopes can be combined with phenotypic analyses of (sub)fossils to test novel hypotheses about past community assembly, and evolutionary processes such as phenotypic change and adaptation, as well as phenotypic diversification and adaptive radiation.

Integration of these new and classical approaches is expanding our ability to understand organism-environment interactions and evolutionary responses to environmental change over time.
