The evolutionary ecology of fatty-acid variation: implications for consumer adaptation and diversification

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Abstract

The nutritional diversity of resources can affect the adaptive evolution of consumer metabolism and consumer diversification. Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) have a high potential to affect consumer fitness, through their widespread effects on reproduction, growth, and survival. However, few studies consider the evolution of fatty acid metabolism within an ecological context. In this review, we first document the extensive diversity in both primary producer and consumer n-3 LC-PUFA distributions among major ecosystems, between habitats, and among species within habitats. We highlight some of the key nutritional contrasts that can shape behavioral and/or metabolic adaptation in consumers, discussing how consumers can evolve in response to the spatial, seasonal, and community-level variation of resource quality. We propose a hierarchical trait-based approach for studying the evolution of consumers' metabolic networks, and review the evolutionary genetic mechanisms underpinning consumer adaptation to n-3 LC-PUFA distributions. In doing so, we consider how the metabolic traits of consumers are hierarchically structured, from cell membrane function to maternal investment, and have strongly environment-dependent expression. Finally, we conclude with an outlook on how studying the metabolic adaptation of consumers within the context of nutritional landscapes can open up new opportunities for understanding evolutionary diversification.

Introduction

Adaptive evolution of consumer metabolism in response to the spatiotemporal variation of dietary resources can contribute to the origin and maintenance of biological diversity. In natural populations, consumers often face mismatches between the dietary supply of, and physiological requirements for, both inorganic elements (e.g. carbon, nitrogen, and phosphorus) and essential organic compounds (e.g. amino acids, lipids, and vitamins). Ecosystem- and habitat-specific differences in the nutritional quality of prey can generate divergent selection, and thereby influence the evolutionary processes underlying ecological speciation and

adaptive radiation (Schluter 2000; Nosil 2012). Moreover, key metabolic adaptations, such as the ability to synthesize essential compounds, can generate ecological opportunities that enable consumer species to transition into novel adaptive zones (Simpson 1945; Simpson 1953).

In natural populations of consumers, metabolic phenotypes in general, and lipid phenotypes in particular, are important components of fitness variation. Lipids are fundamentally important for energy storage, cell membrane structure, and cellular functions (Sunshine and Iruela-Arispe 2017). Within lipids, the omega-3 (n–3) and omega-6 (n–6) polyunsaturated fatty acids (PUFA) are important for somatic development, especially nervous and gonadal tissues (Arts and Kohler 2009; Guo et al. 2016a; Tocher et al. 2019), cognition (McCann and Ames 2005; Cunanne et al. 2009; Hoffman et al. 2009), reproduction (Martin-Creuzburg et al. 2009; Roqueta-Rivera et al. 2010; Chen et al. 2012; Sinendo et al. 2017), and survival (Matsunari et al. 2013; Fuiman and Perez 2015; Kim et al. 2016; Mesa-Rodriguez et al. 2018; Twining et al. 2018a).

Consumers likely face an allocation tradeoff involving their metabolic capacity to synthesize PUFA and their capacity to acquire fatty-acids (FA) from dietary sources. Indeed, the behavioral and metabolic strategies to meet PUFA requirements vary widely across the tree of life (Fig. 1). For example, detritivorous nematodes have a broad capacity to synthesize PUFA from dietary carbohydrates as well as short-chain fatty acid precursors (Watts and Browse 2002; Malcicka et al. 2018). However, all vertebrates and many major groups of invertebrates lack the ability to convert monounsaturated fatty acids (MUFA) such as oleic acid (OA) to n–3 and n–6 PUFA (Kabeya et al. 2018). Therefore, a finch consuming seeds that lack eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n–3) must metabolically derive these compounds from precursors (e.g., α-linolenic acid, ALA, 18:3n–3) through enzymatic conversion processes, including desaturation and elongation (Fig. 1C). However some species of obligate carnivores, such as cats and tuna (Fig. 1E), are entirely unable to convert short-chain (C_{18}) into the long-chain ([?]$C_{20;}$ LC) PUFA, and can only acquire EPA and/or DHA directly from their diet (Rivers et al. 1975; Betancor et al. 2020; Wang et al. 2020). Even those consumers that can synthesize some EPA and/or DHA from the precursor compounds available in low quality food (e.g., ALA), may face high metabolic costs that manifest as reduced population growth rates. For example, *Daphnia*populations grow much slower (or not all) on a diet low in n-3 LC-PUFA (e.g. cyanobacteria) compared to a diet high in n-3 LC-PUFA (e.g.*Nannochloropsis* or *Cryptomonas* ; Fig. 1D; Martin-Creuzburg et al. 2009; Martin-Creuzburg and von Elert 2009).

At the base of food chains there are two fundamental contrasts in n-3 PUFA availability that are particularly relevant for understanding how spatiotemporal variation of resource quality can influence consumer adaptation (Fig. 2). First, aquatic primary producers often contain both EPA and DHA whereas terrestrial primary producers typically only contain shorter chain n-3 fatty acids, such as ALA (Hixson et al. 2015; Twining et al. 2016a; Colombo et al. 2017). As a result, terrestrial consumers are fundamentally more limited by EPA and DHA availability than aquatic consumers, and have evolved numerous adaptations to resolve this nutritional constraint. Second, within aquatic systems, primary producers in marine ecosystems have higher DHA content than in freshwater ecosystems (Fig. 2). This DHA disparity has driven multiple independent cases of consumer metabolic evolution associated with the adaptation from marine to freshwater ecosystems (Ishikawa et al. 2019). More generally, such fundamental nutritional contrasts among ecosystems, as well as others occurring within ecosystems (e.g. among habitats, and prey species), can contribute to evolutionary tradeoffs involving PUFA acquisition and metabolism.

Previous work has documented how PUFA vary in relation to ecosystem type, trophic level, taxonomy, and foraging behavior of species (e.g., Galloway and Winder 2015; Hixson et al. 2015; Colombo et al. 2017; Guo et al. 2017), but not how variation is important for understanding the prevailing diversity of consumer metabolism within and among species. Here, we review the distribution of n-3 LC-PUFA in both primary producers and consumers among major ecosystem (i.e., freshwater, marine, terrestrial), between adjacent habitats within ecosystems (e.g., nearshore-offshore, stream-forests), and among co-occurring prey species within habitats. We discuss how consumers can evolve in response to the spatial, seasonal, and community-level variation of prey quality. In doing so, we consider how the metabolic traits of consumers are hierarchically

structured, from cell membrane function to maternal investment, and have strongly environment-dependent expression. Finally, we discuss the evolutionary genetic mechanisms that underlie the adaptation of consumers to PUFA limitation, and how such metabolic evolution can be an important driver of consumer diversification in ecosystems.

Heterogeneity of fatty acid distribution in nature: implications for consumers

Primary producers vary widely in their fatty acid composition across ecosystems (Fig. 2A-C), but there are some stark contrasts within and among ecosystems (Table S1). For example, vascular land plants, such as angiosperms and gymnosperms, often contain little to no n-3 LC-PUFA, whereas aquatic algae, such as diatoms and cryptophytes, are often laden with both EPA and DHA (Fig. 2A-C). However, a number of non-vascular and semi-aquatic plants, such as mosses, do contain EPA (e.g., Kalacheva et al. 2009; Fig. 2B). Terrestrial primary producers also contain significantly more fatty acids as ALA, the precursor to EPA and DHA, compared to marine primary producers (Fig. 2A; Table S1). In addition to these patterns with n-3 PUFA, terrestrial primary producers typically contain a higher proportion of n-6, such as LIN, relative to n-3 PUFA, like ALA, compared to aquatic primary producers (Hixson et al. 2015), but the reasons for this are unclear. One possible explanation for these patterns is the higher susceptibility of PUFA with more double bonds, and LC-PUFA in particular, to peroxidation (Halliwell and Gutteridge 1985; Mueller 2004; Møller et al. 2007), which is a greater risk in terrestrial environments. Another important and well-documented pattern is that marine primary producers have a significantly higher percentage of fatty acids as EPA compared to either freshwater or terrestrial primary producers (Fig. 2B; Table S1) as well as significantly more fatty acids as DHA compared to terrestrial primary producers (Fig. 2C; Table S1). The reasons for this pattern are also unclear, but might be partly due to EPA and DHA conferring protection against high salinity (Jiang and Chen 1999; Sui et al. 2010).

Within ecosystems, the distribution of PUFA of primary producers is typically attributed to both species differences (Taipale et al. 2013) and environmental conditions (Lang et al. 2011). For example, n-3 LC-PUFA are very abundant across several major groups of Eukaryotic algae (Mühlroth et al. 2013), but are absent in Cyanobacteria (Twining et al. 2016a). However, the composition and content of FAs can also be highly variable among closely related species and individuals of the same species (Lang et al. 2011; Galloway et al. 2012; Taipale et al. 2013; Charette and Derry 2016), possibly due to the strong influence of environmental conditions (Lang et al. 2011). LC-PUFA molecules are particularly unstable due to the susceptibility of their multiple double bonds to oxidation and attack by reactive oxygen species (Shchepinov et al. 2014). For instance, high temperatures increase reaction rates, such that LC-PUFA degrade faster in warm environments (Hixson and Arts 2016). In addition, phospholipids with double bonds, such as those found in PUFA, may help cells maintain membrane fluidity at lower temperatures (homeoviscous adaptation; Sinensky 1974; Feller et al. 2002). Thus, it may be beneficial for organisms to have more LC-PUFA when it is colder and more costly for them to protect LC-PUFA when it is warmer. In algae, n-3 LC-PUFA content is often negatively correlated with both temperature (Hixson and Arts 2016) and light levels (Amini Khoeyi et al. 2012; Hill et al. 2011), and influenced by inorganic nutrient concentration (e.g., Guschina and Harwood 2009; Piepho et al. 2012). At constant temperature and light levels, phosphorus limitation, for example, can decrease overall lipid content but increase n-3 LC-PUFA production, possibly reflecting the need to store lipids until growth conditions improve (Guschina and Harwood 2009). However, when light, temperature, and nutrients are simultaneously manipulated, fatty acid responses can be highly variable across species and systems (e.g., Piepho et al. 2012; Cashman et al. 2013; Guo et al. 2016b).

In consumers, the composition of PUFA reflects both the dietary sources of lipids (e.g., ecosystem origin, prey availability) and the capacity of consumers to metabolise different FA (Fig. 2, Hixson et al. 2015; Guo et al. 2017). Insect species with an early aquatic life stage often contain more n-3 LC-PUFA than those that are exclusively terrestrial (Twining et al. 2018a), and are thus important sources of EPA for insectivores, such as Eastern Phoebes (*Sayornis phoebe*) (Twining et al. 2019). Many consumers acquire PUFA from multiple ecosystems in order to meet their own nutritional requirements. For example, mammalian carnivores can forage on aquatic resources to help increase their intake of DHA relative to linolenic acid (18:2n-6; LIN),

which is an abundant n-6 PUFA in terrestrial primary producers (Koussoroplis et al. 2008). Migratory consumers can accumulate n-3 LC-PUFA from PUFA-rich ecosystems and use them for reproduction and offspring provisioning in more PUFA-depauperate ecosystems (e.g. salmon migrating from the ocean to freshwater streams; Heintz et al. 2004). Indeed, many species that experience wide temporal variation in resource quality often exhibit either plasticity (e.g., Katan et al. 2019) or genetic adaptation (Ishikawa et al. 2019) associated with fatty acid metabolism.

Within ecosystems, consumers often experience contrasting distributions of FA when foraging in multiple adjacent habitats. Within lakes, for example, ecotypes of Eurasian perch (*Perca fluviatilis*) are known to specialize on either littoral macroinvertebrates, which are DHA-poor, or pelagic zooplankton, which have species (e.g. copepods) that are DHA-rich (Fig. 3A). Intriguingly, in spite of the fact that DHA is higher in pelagic prey, littoral perch typically have higher DHA than pelagic perch. This might indicate that perch can thrive on a low-DHA diet (Scharnweber et al., unpublished), via preferential DHA retention (e.g., Hessen and Leu 2006; Heissenberger et al. 2010) and/or DHA synthesis from precursors like ALA (e.g., Buzzi et al. 1996; Bell et al. 2001). In terrestrial systems, Tree Swallows vary widely in their access to aquatic prey (McCarty and Winkler 1999; Stanton et al. 2016; Michelson et al. 2018), which contain substantially more EPA than terrestrial prey (Twining et al. 2018a; Twining et al. 2019; Fig. 3B). Controlled diet studies show that Tree Swallow chicks, which are inefficient at synthesizing EPA and DHA from ALA (Twining et al. 2018b) grow faster, are in better condition, and have increased survival when they consume either more aquatic insects or diets containing more EPA and DHA (Twining et al. 2016b). Because nest sites vary considerably in their distance to aquatic ecosystems, adults might trade-off food quality with quantity when provisioning their young. Although unexplored, this trade-off could select for increased efficiency of ALA to EPA and DHA conversion in populations that breed in drier, upland habitats that have a lower availability of high-quality freshwater prey.

Contrasting distribution of FA can, in some cases, drive adaptive population divergence of consumers. For example, urban and rural populations of Great Tits (*Parus major*) differ not only in their diet (Andersson et al. 2015) and their fatty acid composition (Andersson et al. 2015; Isaksson et al. 2017) but also in their expression of the*Elovl* and *Fads* genes (Watson et al. 2017), which code for the enzymes used to convert ALA and LIN to n-3 and n-6 LC-PUFA, respectively. Specifically, rural tits have higher plasma EPA content while urban tits have plasma higher arachidonic acid (ARA, 20:4n-6) content (Andersson et al. 2015; Isaksson et al. 2017). The n-3 LC-PUFA have anti-inflammatory properties while n-6 LC-PUFA, which are synthesized from their shorter-chain n-6 precursor through the same pathway as n-3 PUFA, have pro-inflammatory properties (Calder et al. 2002). Urban tits experience greater oxidative stress than do rural tits (Isaksson et al. 2017; Watson et al. 2017) and also express *Elovl*and *Fads* at lower rates compared to rural tits (Watson et al. 2017). Thus, urban tits appear to suppress the production of both n-3 and n-6 LC-PUFA in order to reduce inflammation and oxidative damage in a more stressful environment (Watson et al. 2017).

The network and hierarchical structure of fatty acid traits

The above examples illustrate the varied ways in which consumers adapt to the heterogeneous distributions of n-3 LC-PUFA in nature (Fig. 2), including via the evolution of metabolic capacity for biosynthesis of FA and/or the foraging behaviors underlying the dietary acquisition of n-3 LC-PUFA. In light of this complexity, we suggest an integrative approach that includes both investigating the individual enzymes and processes involved in fatty acid synthesis within the metabolic network (Fig. 4; Table 1), and situating these metabolic traits within a hierarchical structure of functional traits leading to fitness variation (Fig. 5; Table 2).

Although all organisms share core metabolic processes for fatty acid synthesis (Fig. 4), consumer species vary widely in capacity to convert: 1) MUFA to PUFA (Module C, Fig. 4), 2) n–6 to n–3 PUFA (Module D, Fig. 4), and 3) C_{18} n-6 and n-3 PUFA to LC-PUFA (Modules E and F, Fig. 4). Each step within the n-3 LC-PUFA biosynthesis pathway is governed by the presence and activity level of particular enzymes, as well as by the presence and expression levels of specific genes. Saturated fatty acids (SFA), such as stearic acid (18:0), are synthesised *de novo* through the fatty acid synthase (*fasn*) and SFA elongase system (Module A, Fig. 4). Stearoyl-CoA desaturase (*Scd*) can then introduce a double bond at the $\Delta 9$ position of the

fatty carbon chain, producing monounsaturated fatty acids, such as oleic acid (OA, 18:1n-9) (Module B, Fig. 4). All eukaryotes appear to be able to synthesize OA. In contrast, the biosynthesis from MUFA (OA; Module C, Fig. 4) to PUFA, with multiple double bonds like linoleic acid (LIN, 18:2n-6), only exists in a limited number of consumers with the methyl-end (ωx) desaturase enzyme, Δ12 desaturase (Blomquist et al. 1991). Most consumers neither possess the related methyl-end desaturase enzyme ([?]15 desaturase) that is necessary to produce ALA from LIN, nor the [?]17 and [?]19 desaturases to convert n-3 LC-PUFA from their n-6 LC-PUFA counterparts (Module D, Fig. 4). These enzymes introduce an additional double bond between the terminal methyl group of a fatty acyl chain and a pre-existing double bond, allowing the synthesis of PUFA from MUFA, and, importantly, n-3 PUFA from n-6 PUFA. The methyl-end desaturases were historically thought to exist only in plants, algae, protists, fungi and a nematode (i.e. *Caenorhabditis elegans*), but a recent study suggests that this gene family also occurs in cnidarians, additional nematode species, lophotrochozoans (molluscs, annelids, rotifers), and arthropods (copepods and at least two species of insects) (Kabeya et al. 2018; Garrido et al. 2019; Kabeya et al. 2020). A much greater number of consumers are able to elongate and desaturate n-6 and n-3 C_{18}PUFA into corresponding n-6 and n-3 LC-PUFA (Modules E and F, Fig. 4). Network modules E and F involve several front-end desaturases as well as fatty acid elongases (elongation of very long-chain fatty acids protein, *Elovl*) and exist, with varying efficiency, in consumers ranging from molluscs and some arthropods (Monroig and Kabeya 2018) to chickens (Gregory and James 2014; Boschetti et al. 2016) and humans (Leonard et al. 2002; Nakamura and Nara 2004), suggesting that these pathways have evolved multiple times.

In light of the complexity of fatty acid metabolic networks, identifying a set of modules and component traits can be a useful approach. As illustrated in Fig. 4, we identify six core modules based on important functional metabolic capacities (Fig. 4A-F, Table 1A), and further break these down into constituent traits that define the reaction rates between specific FA substrates and products (e.g., ALA to EPA conversion capacity and efficiency, Table 1B). There is some value to such simplifications because they reveal broad-scale patterns in metabolic capacity across the tree of life. However, there is also substantial pleiotropy, in that single genes can modify the activity of numerous reaction rates across the overall metabolic network (Table 1B). For example, in many teleosts, *Fads2* gene products can influence conversion rates of LIN to a series of n-6 LC-PUFA including ARA (Fig. 4, Module E), as well as ALA to a series of n-3 LC-PUFA including EPA and DHA (Fig. 4, Module F). Nevertheless, treating both modules and their component pathways as metabolic traits permits us to document heritable variation within metabolic network modules (Box 1), and to identify both the ecological and genetic mechanisms underlying their adaptation. This is an important step for understanding the complex evolution of metabolic networks (Olson-Manning et al. 2012; Watson et al. 2014; Melián et al. 2018) and the role that metabolism plays in evolutionary diversification more broadly.

The metabolic traits we summarize in Table 1 are also embedded within a hierarchy of other potentially fitness-relevant consumer traits (Table 2). Natural selection acts upon the heritable intraspecific metabolic traits in the context of other subordinate and emergent functional traits in the hierarchy (Fig. 5; Henshaw et al. 2020; Laughlin et al. 2020). Where there is a heritable basis for metabolic traits, there is the potential for adaptive evolution of consumer metabolism in response to natural selection. Such evolution might involve fatty acid synthesis and internal regulation, and/or of behavioral traits related to resource acquisition (e.g., selective foraging) and/or life history traits (e.g., migration and phenology) (see references for Table 2; Fig. 5). The evolution of metabolic traits might evolve independently, or as a correlated response to other heritable traits, and culminate in changes in physiological performance, immunocompetence, and cell membrane fluidity (Table 1, Fig. 5). Such trait change has the potential to influence numerous processes ranging from those affecting individual molecules to those affecting an individual's lifetime reproductive Darwinian fitness (Table 2, Fig. 5).

Evolutionary genetic mechanisms of metabolic adaptation

When consumers experience selection for n-3 LC-PUFA synthesis, they can increase enzymatic activity with three different types of genetic processes: 1) gene copy number increases, 2) enzymatic activity changes by amino acid substitutions, and 3) regulatory mutations that increase transcription rates (Fig. 6). These three

mechanisms differ in their effect sizes and pleiotropy (i.e., the number of phenotypic traits influenced by the gene). Copy number increases may have the strongest effects on metabolic processes like fatty acid synthesis (Loehlin and Carroll 2016; Loehlin et al. 2019), but are also likely to have pleiotropic effects on other metabolic processes. This is because an increase in copy number may affect expression in multiple tissues throughout different ontogenetic stages (developmental pleiotropy) and/or they may change the amounts of other organic compounds produced as by-products when enzymes are multifunctional (biochemical pleiotropy) (Fig. 6B). Pleiotropic changes may be neutral, favorable, or unfavorable with respect to fitness. For instance, because n-3 and n-6 fatty acids are elongated and desaturated via the same metabolic pathway (Fig. 4), increased fatty acid desaturase and/or elongase activity may result in increased production of both n-3 or n-6 LC-PUFA, depending on the relative availability of n-3 and n-6 precursors. Amino acid substitutions generally have even more pleiotropic effects than copy number increases (Carroll 2005), but their effect sizes are reported to be smaller in some cases (Loehlin et al. 2019). Regulatory mutations may have relatively strong effects (Loehlin et al. 2019) and enable tissue- or ontogenetic stage-specific expression, but may still be biochemically pleiotropic. Importantly, these three types of mutations often occur together. After gene duplication, these mutations can diverge in both functional amino acid sequences and expression patterns (Ohno 1970; Lynch 2007), becoming more specific (neo-functionalization) and thus reducing pleiotropic effects while still having strong effect sizes (Fig. 6C).

Examples of all three types of genetic mechanisms can be found within the evolution of fatty acid metabolism. Copy number variation in fatty acid desaturase (*Fads*) genes is widely observed in vertebrates (Castro et al. 2012). For instance, Ishikawa et al. (2019) recently found that freshwater threespine stickleback have increased *Fads2* copy number and thus greater capability to synthesize DHA, thereby overcoming the nutritional constraints of freshwater ecosystems. However, increased expression of*Fads2* also results in increased production of n-6 LC-PUFA, such as ARA, in sticklebacks, thus demonstrating a biochemical pleiotropic effect (Ishikawa et al. 2019). In humans, regulatory mutations are known to underlie adaptation to low n-3 LC-PUFA diets (Fumagalli et al. 2015; Ye et al. 2017; Tucci et al. 2018). Derived alleles with higher *Fads1* expression appear to have enabled humans to survive better on cultivated, land plant-derived, and n-3 LC-PUFA-deficient diets, allowing them to expand their distribution (Ameur et al. 2012; Fumagalli et al. 2015; Tucci et al. 2018). In contrast, human populations that consume n-3 LC-PUFA-rich diets with high amounts of fish and meat have the ancestral haplotypes (Amorin et al. 2017). Amino acid changes that alter enzymatic functions can also help consumers adapt to diets that vary in n-3 LC-PUFA content. For example, although zebrafish (*Danio rerio*) have just one copy of *Fads2* , they have high [?]5 and [?]6 desaturase activities as a result of amino acid changes (Hastings et al. 2001). Neo-functionalization following duplication appears to be a common genetic process (Ohno 1970; Zhang 2003). In fishes, for instance, the acquisition of [?]4 activity occurred in one copy of *Fads2* after gene duplication (Li et al. 2010; Morais et al. 2012; Oboh et al. 2017). Further genetic analysis of variation in fatty acid metabolism across a greater diversity of taxa will help us to understand which mechanisms are the most prevalent and how mechanisms differ in their effect sizes and pleiotropy on fatty acid adaptive landscapes.

Metabolic adaptation and consumer diversification

The network structure of fatty acid metabolism, including the modularity and degree of pleiotropy, has important consequences for understanding how organisms evolve and diversify over time. For instance, while the primary photosynthetic pathways of plants are highly conserved, some of its components have diversified widely, culminating in lineage-specific pathway regulation and structure (Maeda 2019). As a consequence of network structure, evolutionary changes at early steps within photosynthesis can have more substantial effects on final products than those that occur in later steps (Kacser and Burns 1981; Wright and Rausher 2010; Olson-Manning et al. 2012). In consumers, the evolution of metabolic networks has allowed them to utilize new resources or synthesize essential organic compounds that were previously required from diet (Borenstein et al. 2008; Wagner 2012). Within a lineage, species can differ in the number and connectivity of modules in metabolic networks as well as in synthesis activities across the network. The evolution of carotenoid networks in birds, for example, has led to considerable variation in the structure (i.e., gain and loss of modules) and connectivity of functional modules (Morrison and Badyaev 2016), and, interestingly,

has been implicated in the diversification of avian color patterns (Badyaev et al. 2019a).

In the 1940s, Simpson posited that species could enter new 'adaptive zones' (Simpson 1945; Simpson 1953) via specific events, including dispersal into new habitats, extirpation of predators, or through 'key innovations', namely those that relax or fundamentally change the prevailing environmental sources of natural selection (Miller 1949; Rabosky 2017). While microevolutionary dynamics might shape the existing structure or control of metabolic networks (Figs. 4-5), large structural changes in the network itself, such as the internalization of an external dependency (e.g., the ability to synthesize a formerly essential dietary fatty acid), might present a species with novel ecological opportunity. In other words, the evolution of fatty acid metabolism might afford species new opportunities to exploit novel resources (e.g. terrestrial plants containing only ALA), and allow them to persist and diversify in 'adaptive zones'. For example, freshwater threespine stickleback have reduced their external dependency on DHA-rich resources, which are limited outside of marine habitats (Fig. 3A), by increasing their endogenous conversion rates from ALA to DHA, a key innovation. Badyaev et al. (2019a) propose that such evolution in the control of metabolic networks is fundamentally associated with macroscale patterns of species diversity. Specifically, local metabolic adaptation can culminate in shifts in network topologies, potentially opening new opportunities for evolutionary diversity (Badyaev 2019b). Currently, this is unexplored in the context of fatty-acid metabolism, but there is considerable potential to do so in light of the heterogeneity of FA within and among ecosystems (Fig. 2), genetic variation and fitness relevance of FA acquisition and metabolism traits, and examples of key innovations facilitating consumer diversification (Ishikawa et al. 2019).

Conclusion

There is a global metabolic network available for animals (Borenstein et al. 2008), and we are still in the early stages of uncovering how this network is structured across the animal tree of life (Fig. 1, Fig. 4, Table 1) and how it is mechanistically linked to fitness variation of consumers (Table 2, Fig. 5). Empirical studies have documented substantial variation in the fatty acids of organisms, structured both within and among ecosystems and among prey communities, which creates ample opportunities for the evolution of consumer behavior and metabolism. Indeed, there is growing evidence for evolutionary diversification in the primary nodes and controls of the fatty acid metabolic network (Fig. 4). Studying such metabolic diversity across multiple scales (Fig. 4, 5), will allow us to understand more broadly how consumers evolve traits related to both resource acquisition and metabolism, and how they invade new environments and diversify.

References

1.

Ameur, A., Enroth, S., Johansson, A., Zaboli, G., Igl, W., Johansson, Anna C.V. *et al.* (2012). Genetic Adaptation of Fatty-Acid Metabolism: A Human-Specific Haplotype Increasing the Biosynthesis of Long-Chain Omega-3 and Omega-6 Fatty Acids. *The American Journal of Human Genetics* , 90, 809-820.

2.

Amini Khoeyi, Z., Seyfabadi, J. & Ramezanpour, Z. (2012). Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris. *Aquac. Int.* , 20, 41-49.

3.

Amorim, C.E.G., Nunes, K., Meyer, D., Comas, D., Bortolini, M.C., Salzano, F.M. *et al.* (2017). Genetic signature of natural selection in first Americans. *Proceedings of the National Academy of Sciences* , 114, 2195-2199.

4.

Andersson, M.N., Wang, H.-L., Nord, A., Salmon, P. & Isaksson, C. (2015). Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Frontiers in Ecology and Evolution* , 3.

5.

Arts, M.T. & Kohler, C.C. (2009). Health and condition in fish: the influence of lipids on membrane competency and immune response. In:*Lipids in Aquatic Ecosystems* (eds. Kainz, M, Brett, MT & Arts, MT). Springer New York New York, NY, pp. 237-256.

6.

Badyaev, A.V. (2019). Evolutionary transitions in controls reconcile adaptation with continuity of evolution. *Semin. Cell Dev. Biol.* , 88, 36-45.

7.

Badyaev, A.V., Posner, A.B., Morrison, E.S. & Higginson, D.M. (2019). Cycles of external dependency drive evolution of avian carotenoid networks. *Nat. Commun.* , 10, 1596.

8.

Barrett, R.D. & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in ecology & evolution* , 23, 38-44.

9.

Barton, N.H. & Keightley, P.D. (2002). Understanding quantitative genetic variation. *Nature Reviews Genetics* , 3, 11-21.

10.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *The Journal of nutrition* , 131, 1535-1543.

11.

Betancor, M.B., Oboh, A., Ortega, A., Mourente, G., Navarro, J.C., de la Gandara, F. *et al.* (2020). Molecular and functional characterisation of a putative elovl4 gene and its expression in response to dietary fatty acid profile in Atlantic bluefin tuna (Thunnus thynnus). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* , 240, 110372.

12.

Bickel, R.D., Kopp, A. & Nuzhdin, S.V. (2011). Composite effects of polymorphisms near multiple regulatory elements create a major-effect QTL. *PLoS Genet* , 7, e1001275.

13.

Blomquist, G.J., Borgeson, C.E. & Vundla, M. (1991). Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochem.* , 21, 99-106.

14.

Borenstein, E., Kupiec, M., Feldman, M.W. & Ruppin, E. (2008). Large-scale reconstruction and phylogenetic analysis of metabolic environments. *Proc. Natl. Acad. Sci. U. S. A.* , 105, 14482-14487.

15.

Boschetti, E., Bordoni, A., Meluzzi, A., Castellini, C., Dal Bosco, A. & Sirri, F. (2016). Fatty acid composition of chicken breast meat is dependent on genotype-related variation of FADS1 and FADS2 gene expression and desaturating activity. *Animal* , 10, 700-708.

16.

Buzzi, M., Henderson, R.J. & Sargent, J.R. (1996). The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (Oncorhynchus mykiss) fed diets containing fish oil or olive oil. *Biochim Biophys Acta* , 1299, 235-244.

17.

Calder, P.C. (2002). Dietary modification of inflammation with lipids.*Proceedings of the Nutrition Society* , 61, 345-358.

18.

Carroll, S.B. (2005). Evolution at two levels: on genes and form.*PLoS Biol.* , 3, e245.

19.

Cashman, M.J., Wehr, J.D. & Truhn, K. (2013). Elevated light and nutrients alter the nutritional quality of stream periphyton.*Freshwater Biology* , 58, 1447-1457.

20.

Castro, L.F.C., Monroig, O., Leaver, M.J., Wilson, J., Cunha, I. & Tocher, D.R. (2012). Functional desaturase Fads1 (Δ5) and Fads2 (Δ6) orthologues evolved before the origin of jawed vertebrates. *PLoS One* , 7, e31950.

21.

Cesar, A.S., Regitano, L.C., Mourão, G.B., Tullio, R.R., Lanna, D.P., Nassu, R.T. *et al.* (2014). Genome-wide association study for intramuscular fat deposition and composition in Nellore cattle.*BMC genetics* , 15, 39.

22.

Chaguaceda, F., Eklöv, P. & Scharnweber, K. (2020). Regulation of fatty acid composition related to ontogenetic changes and niche differentiation of a common aquatic consumer. *Oecologia* , 193, 325-336.

23.

Charette, C. & Derry, A.M. (2016). Climate alters intraspecific variation in copepod effect traits through pond food webs.*Ecology* , 97, 1239-1250.

24.

Chen, M., Liu, H. & Chen, B. (2012). Effects of dietary essential fatty acids on reproduction rates of a subtropical calanoid copepod, Acartia erythraea. *Mar. Ecol. Prog. Ser.* , 455, 95-110.

25.

Colombo, S.M., Wacker, A., Parrish, C.C., Kainz, M.J. & Arts, M.T. (2017). A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems.*Environ. Rev.* , 25, 163-174.

26.

Cunnane, S.C., Plourde, M., Pifferi, F., Bégin, M., Féart, C. & Barberger-Gateau, P. (2009). Fish, docosahexaenoic acid and Alzheimer's disease. *Prog. Lipid Res.* , 48, 239-256.

27.

Feller, S.E., Gawrisch, K. & MacKerell, A.D. (2002). Polyunsaturated fatty acids in lipid bilayers: intrinsic and environmental contributions to their unique physical properties. *Journal of the American Chemical Society* , 124, 318-326.

28.

Fuiman, L.A. & Perez, K.O. (2015). Metabolic programming mediated by an essential fatty acid alters body composition and survival skills of a marine fish. *Proceedings of the Royal Society B: Biological Sciences* , 282, 20151414.

29.

Fumagalli, M., Moltke, I., Grarup, N., Racimo, F., Bjerregaard, P., Jørgensen, M.E. *et al.* (2015). Greenlandic Inuit show genetic signatures of diet and climate adaptation. *Science* , 349, 1343-1347.

30.

Galloway, A.W.E., Britton-Simmons, K.H., Duggins, D.O., Gabrielson, P.W. & Brett, M.T. (2012). Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. *J. Phycol.* , 48, 956-965.

31.

Galloway, A.W.E. & Winder, M. (2015). Partitioning the Relative Importance of Phylogeny and Environmental Conditions on Phytoplankton Fatty Acids. *PLoS ONE* , 10, e0130053.

32.

Garrido, D., Kabeya, N., Hontoria, F., Navarro, J.C., Reis, D.B., Martín, M.V. *et al.* (2019). Methyl-end desaturases with [?]12 and ω3 regioselectivities enable the de novo PUFA biosynthesis in the cephalopod Octopus vulgaris. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* , 1864, 1134-1144.

33.

Gregory, M.K. & James, M.J. (2014). Functional characterization of the duck and turkey fatty acyl elongase enzymes ELOVL5 and ELOVL2. *J. Nutr.* , 144, 1234-1239.

34.

Guo, F., Bunn, S.E., Brett, M.T. & Kainz, M.J. (2017). Polyunsaturated fatty acids in stream food webs – high dissimilarity among producers and consumers. *Freshwater Biology* , 62, 1325-1334.

35.

Guo, F., Kainz, M.J., Sheldon, F. & Bunn, S.E. (2016). The importance of high-quality algal food sources in stream food webs – current status and future perspectives. *Freshwater Biology* , 61, 815-831.

36.

Guo, F., Kainz, M.J., Sheldon, F. & Bunn, S.E. (2016). Effects of light and nutrients on periphyton and the fatty acid composition and somatic growth of invertebrate grazers in subtropical streams. *Oecologia* , 181, 449-462.

37.

Guschina, I.A. & Harwood, J.L. (2009). Algal lipids and effect of the environment on their biochemistry. In: *Lipids in aquatic ecosystems* . Springer, pp. 1-24.

38.

Halliwell, B. & Gutteridge, J.M.C. (1985). Free radicals in biology and medicine.

39.

Hastings, N., Agaba, M., Tocher, D.R., Leaver, M.J., Dick, J.R., Sargent, J.R. *et al.* (2001). A vertebrate fatty acid desaturase with Delta 5 and Delta 6 activities. *Proc. Natl. Acad. Sci. U. S. A.* , 98, 14304-14309.

40.

Heintz, R.A., Nelson, B.D., Hudson, J., Larsen, M., Holland, L. & Wipfli, M. (2004). Marine subsidies in freshwater: Effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. *Transactions of the American Fisheries Society* , 133, 559-567.

41.

Heissenberger, M., Watzke, J. & Kainz, M.J. (2010). Effect of nutrition on fatty acid profiles of riverine, lacustrine, and aquaculture-raised salmonids of pre-alpine habitats. *Hydrobiologia* , 650, 243-254.

42.

Henshaw, J.M., Morrissey, M.B. & Jones, A.G. 2020. Quantifying the causal pathways contributing to natural selection. *Evolution* .

43.

Hessen, D.O. & Leu, E. (2006). Trophic transfer and trophic modification of fatty acids in high Arctic lakes. *Freshwater Biology* , 51, 1987-1998.

44.

Hill, W.R., Rinchard, J. & Czesny, S. (2011). Light, nutrients and the fatty acid composition of stream periphyton. *Freshw. Biol.* , 56, 1825-1836.

45.

Hixson, S.M. & Arts, M.T. (2016). Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Glob. Chang. Biol.* , 22, 2744-2755.

46.

Hixson, S.M., Sharma, B., Kainz, M.J., Wacker, A. & Arts, M.T. (2015). Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. *Environmental Reviews* , 23, 414-424.

47.

Hoffman, D.R., Boettcher, J.A. & Diersen-Schade, D.A. (2009). Toward optimizing vision and cognition in term infants by dietary docosahexaenoic and arachidonic acid supplementation: a review of randomized controlled trials. *Prostaglandins Leukot. Essent. Fatty Acids* , 81, 151-158.

48.

Horn, S.S., Ruyter, B., Meuwissen, T.H., Moghadam, H., Hillestad, B. & Sonesson, A.K. (2020). GWAS identifies genetic variants associated with omega-3 fatty acid composition of Atlantic salmon fillets.*Aquaculture* , 514, 734494.

49.

Hu, S., Wang, J., Han, T., Li, X., Jiang, Y. & Wang, C. (2017). Effects of dietary DHA/EPA ratios on growth performance, survival and fatty acid composition of juvenile swimming crab (Portunus trituberculatus).*Aquac. Res.* , 48, 1291-1301.

50.

Isaksson, C., Andersson, M.N., Nord, A., von Post, M. & Wang, H.-L. (2017). Species-dependent effects of the urban environment on fatty acid composition and oxidative stress in birds. *Frontiers in Ecology and Evolution* , 5.

51.

Ishikawa, A., Kabeya, N., Ikeya, K., Kakioka, R., Cech, J.N., Osada, N.*et al.* (2019). A key metabolic gene for recurrent freshwater colonization and radiation in fishes. *Science* , 364, 886-889.

52.

Jiang, Y. & Chen, F. (1999). Effects of salinity on cell growth and docosahexaenoic acid content of the heterotrophic marine microalga Crypthecodinium cohnii. *J. Ind. Microbiol. Biotechnol.* , 23, 508-513.

53.

Kabeya, N., Fonseca, M.M., Ferrier, D.E.K., Navarro, J.C., Bay, L.K., Francis, D.S. *et al.* (2018). Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci Adv* , 4, eaar6849.

54.

Kabeya, N., Gür, İ., Oboh, A., Evjemo, J.O., Malzahn, A.M., Hontoria, F.*et al.* (2020). Unique fatty acid desaturase capacities uncovered in Hediste diversicolor illustrate the roles of aquatic invertebrates in trophic upgrading. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* , 375, 20190654.

55.

Kacser, H. & Burns, J.A. (1981). The molecular basis of dominance.*Genetics* , 97, 639-666.

56.

Kalacheva, G.S., Sushchik, N.N., Gladyshev, M.I. & Makhutova, O.N. (2009). Seasonal dynamics of fatty acids in the lipids of water moss Fontinalis antipyretica from the Yenisei River. *Russ. J. Plant Physiol.* , 56, 795-807.

57.

Katan, T., Caballero-Solares, A., Taylor, R.G., Rise, M.L. & Parrish, C.C. (2019). Effect of plant-based diets with varying ratios of omega 6 to omega 3 fatty acids on growth performance, tissue composition, fatty acid biosynthesis and lipid-related gene expression in Atlantic salmon (Salmo solar). *Comp. Biochem. Physiol. D-Genomics Proteomics* , 30, 290-304.

58.

Kelly, M., Tume, R., Fortes, M. & Thompson, J. (2014). Whole-genome association study of fatty acid composition in a diverse range of beef cattle breeds. *Journal of Animal Science* , 92, 1895-1901.

59.

Kim, J., Yin, T., Shinozaki, K., Lampe, J.W. & Becker, L.B. (2016). DHA-supplemented diet increases the survival of rats following asphyxia-induced cardiac arrest and cardiopulmonary bypass resuscitation. *Sci. Rep.* , 6, 36545.

60.

Koussoroplis, A.-M., Lemarchand, C., Bec, A., Desvilettes, C., Amblard, C., Fournier, C. *et al.* (2008). From Aquatic to Terrestrial Food Webs: Decrease of the Docosahexaenoic Acid/Linoleic Acid Ratio.*Lipids* , 43, 461-466.

61.

Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution* , 1210-1226.

62.

Lang, I., Hodac, L., Friedl, T. & Feussner, I. (2011). Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection.*BMC Plant Biol.* , 11, 124.

63.

Laughlin, D.C., Gremer, J.R., Adler, P.B., Mitchell, R.M. & Moore, M.M. (2020). The Net Effect of Functional Traits on Fitness. *Trends in Ecology & Evolution* .

64.

Lee, Y.W., Gould, B.A. & Stinchcombe, J.R. (2014). Identifying the genes underlying quantitative traits: a rationale for the QTN programme.*AoB Plants* , 6.

65.

Lemos, M.V., Chiaia, H.L.J., Berton, M.P., Feitosa, F.L., Aboujaoud, C., Camargo, G.M. *et al.* (2016). Genome-wide association between single nucleotide polymorphisms with beef fatty acid profile in Nellore cattle using the single step procedure. *BMC genomics* , 17, 213.

66.

Leonard, A.E., Kelder, B., Bobik, E.G., Chuang, L.-T., Lewis, C.J., Kopchick, J.J. *et al.* (2002). Identification and expression of mammalian long-chain PUFA elongation enzymes. *Lipids* , 37, 733-740.

67.

Li, Y., Monroig, O., Zhang, L., Wang, S., Zheng, X., Dick, J.R. *et al.* (2010). Vertebrate fatty acyl desaturase with Δ4 activity.*Proc. Natl. Acad. Sci. U. S. A.* , 107, 16840-16845.

68.

Lin, G., Wang, L., Te Ngoh, S., Ji, L., Orbán, L. & Yue, G.H. (2018). Mapping QTL for omega-3 content in hybrid saline tilapia. *Marine biotechnology* , 20, 10-19.

69.

Loehlin, D.W., Ames, J.R., Vaccaro, K. & Carroll, S.B. (2019). A major role for noncoding regulatory mutations in the evolution of enzyme activity. *Proc. Natl. Acad. Sci. U. S. A.* , 116, 12383-12389.

70.

Loehlin, D.W. & Carroll, S.B. (2016). Expression of tandem gene duplicates is often greater than twofold. *Proc. Natl. Acad. Sci. U. S. A.* , 113, 5988-5992.

71.

Lynch, M. (2007). *The Origins of Genome Architecture* . Sinauer Associates.

72.

Lynch, M. & Walsh, B. (1998). *Genetics and analysis of quantitative traits* . Sinauer Sunderland, MA.

73.

Maeda, H.A. (2019). Evolutionary Diversification of Primary Metabolism and Its Contribution to Plant Chemical Diversity. *Front. Plant Sci.* , 10, 881.

74.

Malcicka, M., Visser, B. & Ellers, J. (2018). An Evolutionary Perspective on Linoleic Acid Synthesis in Animals. *Evol. Biol.* , 45, 15-26.

75.

Martin-Creuzburg, D., Sperfeld, E. & Wacker, A. (2009). Colimitation of a freshwater herbivore by sterols and polyunsaturated fatty acids.*Proc. Biol. Sci.* , 276, 1805-1814.

76.

Martin-Creuzburg, D. & von Elert, E. (2009). Good food versus bad food: the role of sterols and polyunsaturated fatty acids in determining growth and reproduction of Daphnia magna. *Aquatic Ecology* , 43, 943-950.

77.

Matsunari, H., Hashimoto, H., Oda, K., Masuda, Y., Imaizumi, H., Teruya, K. *et al.* (2013). Effects of docosahexaenoic acid on growth, survival and swim bladder inflation of larval amberjack (Seriola dumerili, Risso). *Aquac. Res.* , 44, 1696-1705.

78.

McCann, J.C. & Ames, B.N. (2005). Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am. J. Clin. Nutr.* , 82, 281-295.

79.

McCarty, J.P. & Winkler, D.W. (1999). Foraging Ecology and Diet Selectivity of Tree Swallows Feeding Nestlings. *Condor* , 101, 246-254.

80.

Melián, C.J., Matthews, B., de Andreazzi, C.S., Rodríguez, J.P., Harmon, L.J. & Fortuna, M.A. (2018). Deciphering the Interdependence between Ecological and Evolutionary Networks. *Trends Ecol. Evol.* , 33, 504-512.

81.

Mesa-Rodriguez, A., Maria Hernandez-Cruz, C., Beatriz Betancor, M., Fernandez-Palacios, H., Izquierdo, M.S. & Roo, J. (2018). Effect of increasing docosahexaenoic acid content in weaning diets on survival, growth and skeletal anomalies of longfin yellowtail (Seriola rivoliana, Valenciennes 1833). *Aquac. Res.* , 49, 1200-1209.

82.

Michelson, C.I., Clark, R.G. & Morrissey, C.A. (2018). Agricultural land cover does not affect the diet of Tree Swallows in wetland-dominated habitats. *Condor* , 120, 751-764.

83.

Miller, A.H. (1949). Some ecologic and morphologic considerations in the evolution of higher taxonomic categories. *Ornithologie als biologische Wissenschaft* , 84-88.

84.

Møller, I.M., Jensen, P.E. & Hansson, A. (2007). Oxidative Modifications to Cellular Components in Plants. *Annu. Rev. Plant Biol.* , 58, 459-481.

85.

Monroig, Ó. & Kabeya, N. (2018). Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. *Fish. Sci.* , 84, 911-928.

86.

Morais, S., Castanheira, F., Martinez-Rubio, L., Conceição, L.E.C. & Tocher, D.R. (2012). Long chain polyunsaturated fatty acid synthesis in a marine vertebrate: ontogenetic and nutritional regulation of a fatty acyl desaturase with Δ4 activity. *Biochim. Biophys. Acta* , 1821, 660-671.

87.

Morrison, E.S. & Badyaev, A.V. (2016). Structuring evolution: biochemical networks and metabolic diversification in birds. *BMC Evol. Biol.* , 16, 168.

88.

Mueller, M.J. (2004). Archetype signals in plants: the phytoprostanes.

Curr. Opin. Plant Biol.

89.

Mühlroth, A., Li, K., Røkke, G., Winge, P., Olsen, Y., Hohmann-Marriott, M.F. *et al.* (2013). Pathways of lipid metabolism in marine algae, co-expression network, bottlenecks and candidate genes for enhanced production of EPA and DHA in species of Chromista. *Mar. Drugs* , 11, 4662-4697.

90.

Nakamura, M.T. & Nara, T.Y. (2004). Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu. Rev. Nutr.* , 24, 345-376.

91.

Nosil, P. (2012). *Ecological Speciation* . Oxford University Press.

92.

Oboh, A., Kabeya, N., Carmona-Antoñanzas, G., Castro, L.F.C., Dick, J.R., Tocher, D.R. *et al.* (2017). Two alternative pathways for docosahexaenoic acid (DHA, 22:6n-3) biosynthesis are widespread among teleost fish. *Scientific Reports* , 7.

93.

Ohno, S. (1970). *Evolution by Gene Duplication* . Springer, Berlin, Heidelberg.

94.

Olson-Manning, C.F., Wagner, M.R. & Mitchell-Olds, T. (2012). Adaptive evolution: evaluating empirical support for theoretical predictions.*Nat. Rev. Genet.* , 13, 867-877.

95.

Orr, H.A. (2005). The genetic theory of adaptation: a brief history.*Nature Reviews Genetics* , 6, 119-127.

96.

Piepho, M., Arts, M.T. & Wacker, A. (2012). Species-specific variation in fatty acid concentrations of four phytoplankton species: does phosphorus supply influence the effect of light intensity or temperature? 1. *J. Phycol.* , 48, 64-73.

97.

Rabosky, D.L. (2017). Phylogenetic tests for evolutionary innovation: the problematic link between key innovations and exceptional diversification. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* , 372.

98.

Remington, D.L. (2015). Alleles versus mutations: Understanding the evolution of genetic architecture requires a molecular perspective on allelic origins. *Evolution* , 69, 3025-3038.

99.

Rivers, J.P., Sinclair, A.J. & Craqford, M.A. (1975). Inability of the cat to desaturate essential fatty acids. *Nature* , 258, 171-173.

100.

Roqueta-Rivera, M., Stroud, C.K., Haschek, W.M., Akare, S.J., Segre, M., Brush, R.S. *et al.* (2010). Docosahexaenoic acid supplementation fully restores fertility and spermatogenesis in male delta-6 desaturase-null mice. *J. Lipid Res.* , 51, 360-367.

101.

Schaeffer, L., Gohlke, H., Müller, M., Heid, I.M., Palmer, L.J., Kompauer, I. *et al.* (2006). Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Human molecular genetics* , 15, 1745-1756.

102.

Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution* , 50, 1766-1774.

103.

Schluter, D. (2000). *The ecology of adaptive radiation* . OUP Oxford.

104.

Shchepinov, M.S., Roginsky, V.A., Brenna, J.T., Molinari, R.J., To, R., Tsui, H. *et al.* (2014). Chapter 31 - Deuterium Protection of Polyunsaturated Fatty Acids against Lipid Peroxidation: A Novel Approach to Mitigating Mitochondrial Neurological Diseases. In: *Omega-3 Fatty Acids in Brain and Neurological Health* (eds. Watson, RR & De Meester, F). Academic Press Boston, pp. 373-383.

105.

Simpson, G.G. (1945). Tempo and mode in evolution. *Trans. N. Y. Acad. Sci.* , 8, 45-60.

106.

Simpson, G.G. (1953). The Major Features of Evolution.

107.

Sinedino, L.D.P., Honda, P.M., Souza, L.R.L., Lock, A.L., Boland, M.P., Staples, C.R. *et al.* (2017). Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. *Reproduction* , 153, 707-723.

108.

Sinensky, M. (1974). Homeoviscous adaptation–a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli.*Proceedings of the National Academy of Sciences of the United States of America* , 71, 522-525.

109.

Stanton, R.L., Morrissey, C.A. & Clark, R.G. (2016). Tree Swallow (Tachycineta bicolor) foraging responses to agricultural land use and abundance of insect prey. *Can. J. Zool.* , 94, 637-642.

110.

Stern, D.L. & Frankel, N. (2013). The structure and evolution of cis-regulatory regions: the shavenbaby story. *Philosophical Transactions of the Royal Society B: Biological Sciences* , 368, 20130028.

111.

Sui, N., Li, M., Li, K., Song, J. & Wang, B.S. (2010). Increase in unsaturated fatty acids in membrane lipids of Suaeda salsa L. enhances protection of photosystem II under high salinity.*Photosynthetica* , 48, 623-629.

112.

Sunshine, H. & Iruela-Arispe, M.L. (2017). Membrane lipids and cell signaling. *Curr Opin Lipidol* , 28, 408-413.

113.

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A.W.E., Ojala, A. & Brett, M.T. (2013). Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquat. Microb. Ecol.* , 71, 165-178.

114.

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E. & Napier, J.A. (2019). Omega-3 Long-Chain Polyunsaturated Fatty Acids, EPA and DHA: Bridging the Gap between Supply and Demand. *Nutrients* , 11.

115.

Tucci, S., Vohr, S.H., McCoy, R.C., Vernot, B., Robinson, M.R., Barbieri, C. *et al.* (2018). Evolutionary history and adaptation of a human pygmy population of Flores Island, Indonesia. *Science* , 361, 511-516.

116.

Twining, C.W., Brenna, J.T., Hairston, N.G., Jr. & Flecker, A.S. (2016). Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos* , 125, 749-760.

117.

Twining, C.W., Brenna, J.T., Lawrence, P., Shipley, J.R., Tollefson, T.N. & Winkler, D.W. (2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity.*Proc. Natl. Acad. Sci. U. S. A.* , 113, 10920-10925.

118.

Twining, C.W., Brenna, J.T., Lawrence, P., Winkler, D.W., Flecker, A.S. & Hairston, N.G., Jr. (2019). Aquatic and terrestrial resources are not nutritionally reciprocal for consumers. *Funct. Ecol.* , 33, 2042-2052.

119.

Twining, C.W., Lawrence, P., Winkler, D.W., Flecker, A.S. & Brenna, J.T. (2018). Conversion efficiency of α-linolenic acid to omega-3 highly unsaturated fatty acids in aerial insectivore chicks. *J. Exp. Biol.* , 221.

120.

Twining, C.W., Shipley, J.R. & Winkler, D.W. (2018). Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird.*Ecol. Lett.* , 21, 1812-1820.

121.

Wagner, A. (2012). Metabolic networks and their evolution. *Adv. Exp. Med. Biol.* , 751, 29-52.

122.

Wang, S., Wang, M., Zhang, H., Yan, X., Guo, H., You, C. *et al.*(2020). Long-chain polyunsaturated fatty acid metabolism in carnivorous marine teleosts: Insight into the profile of endogenous biosynthesis in golden pompano Trachinotus ovatus. *Aquac. Res.* , 51, 623-635.

123.

Watson, H., Videvall, E., Andersson, M.N. & Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Sci Rep* , 7, 44180.

124.

Watson, R.A., Wagner, G.P., Pavlicev, M., Weinreich, D.M. & Mills, R. (2014). The evolution of phenotypic correlations and "developmental memory". *Evolution* , 68, 1124-1138.

125.

Watts, J.L. & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U. S. A.* , 99, 5854-5859.

126.

Wright, K.M. & Rausher, M.D. (2010). The evolution of control and distribution of adaptive mutations in a metabolic pathway.*Genetics* , 184, 483-502.

127.

Xia, J.H., Lin, G., He, X., Yunping, B., Liu, P., Liu, F. *et al.*(2014). Mapping quantitative trait loci for omega-3 fatty acids in Asian seabass. *Marine biotechnology* , 16, 1-9.

128.

Ye, K., Gao, F., Wang, D., Bar-Yosef, O. & Keinan, A. (2017). Dietary adaptation of FADS genes in Europe varied across time and geography.*Nat Ecol Evol* , 1, 167.

129.

Zhang, J. (2003). Evolution by gene duplication: an update. *Trends Ecol. Evol.* , 18, 292-298.

130.

Zhang, W., Zhang, J., Cui, L., Ma, J., Chen, C., Ai, H. *et al.*(2016). Genetic architecture of fatty acid composition in the longissimusdorsi muscle revealed by genome-wide association studies on diverse pig populations. *Genetics Selection Evolution* , 48, 5.

Figures, Box, and Tables

Fig. 1: Consumers differ in their capacity to synthesize key fatty acids from precursors. Three of the major gaps in synthesis capacity (A) include: 1) conversion of saturated fatty acids (SFA), which may be derived from carbohydrates in diet, to the monounsaturated fatty acid (MUFA) oleic acid (18:1n-9; OA) and the omega-6 (n-6) polyunsaturated fatty acid (PUFA) linoleic acid (18:2n-6; LIN), 2) conversion of the n-6 PUFA LIN and arachidonic acid (20:4n-6; ARA) to the omega-3 PUFA alpha-linolenic acid (18:3n-3; ALA) and eicosapentaenoic acid (20:5n-3; EPA), respectively, and 3) conversion of the short-chain n-3 PUFA ALA into the long-chain n-3 PUFA EPA and docosahexaenoic acid (22:6n-3; DHA). Within primary producers (A), vascular terrestrial plants are only capable of synthesizing fatty acids up to ALA whereas different species of algae and non-vascular terrestrial plants (e.g., mosses and liverworts) are also able to produce EPA and/or DHA. Consumers (B-E) have evolved synthesis capabilities that differ based upon the availability of key fatty acids in their diets. While some consumers (B) are capable of synthesizing both short-chain and long-chain n-3 and n-6 PUFA from SFA and OA, others (C-D) require short-chain n-3 PUFA from diet, and still others (E) must receive all key fatty acids directly from diet. Some animals, such as soil nematodes (B), consume PUFA-deficient resources like bacteria and organic matter and derive only SFA and OA from their diet, which they use as precursors to synthesize LA, ARA, ALA, EPA, and DHA. Others, such as finches and other terrestrial birds (C), consume resources like seeds that contain only SFA, OA, and LIN, as well as resources like terrestrial insects that also contain ALA. They must therefore convert dietary LIN into ARA and dietary ALA into EPA and DHA. Still others, like Daphnia and other aquatic invertebrates (D), consume

some resources that contain both short-chain and long-chain PUFA, and are incapable of converting EPA to DHA, but are also capable of synthesizing EPA from DHA, such as from the Cryptophyte alga Cryptomonas, through the process of beta-oxidation (BETA). Finally, some animals, like tuna (E) and other carnivorous marine fishes, consume resources that contain the full set of key fatty acids, including both EPA and DHA, and are unable to perform any of the major synthesis steps in (A).

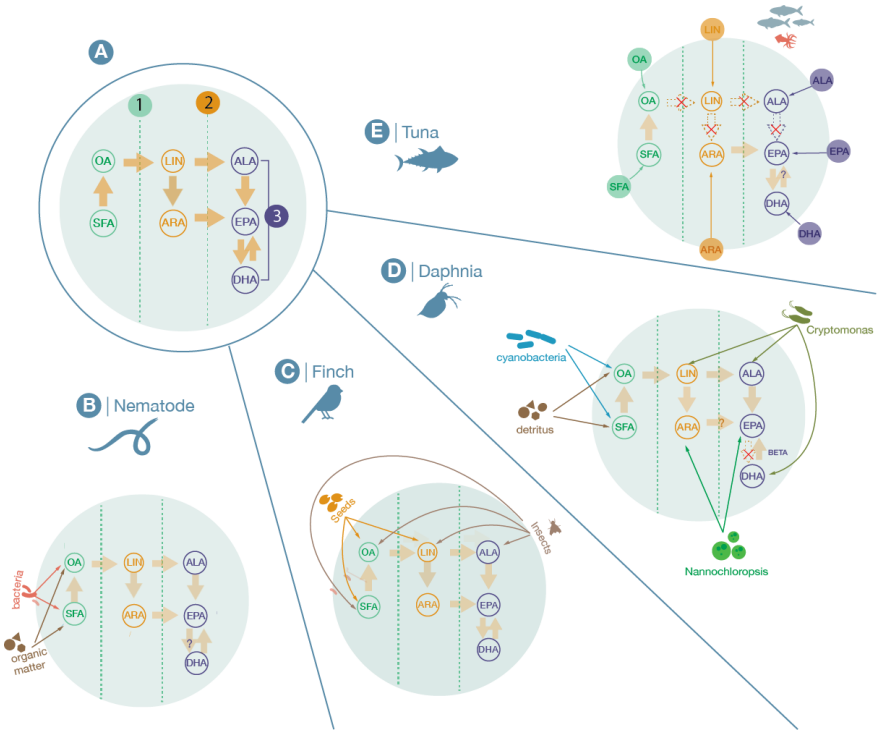

Fig. 2: Marine, freshwater, and terrestrial organisms differ in their fatty acid composition, especially in terms of the omega-3 PUFA ALA, EPA, and DHA. These ecosystem-based differences are most pronounced in (A-C) primary producers, but also occur among (E-F) higher order consumers. (A-C) Vascular terrestrial primary producers contain only the shorter-chain omega-3 PUFA ALA, while aquatic primary producers, as well as a few non-vascular terrestrial primary producers like Bryophytes, also contain the longer-chain omega-3 PUFA EPA and/or DHA. (D-F) Consumers contain more EPA and/or DHA than primary producers from the same ecosystem, but also exhibit differences based on both ecosystem (e.g., terrestrial versus marine mammals and terrestrial versus freshwater insects) and trophic position (e.g., fish versus insects or Cladocerans).

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Fig. 3: A) The available prey of Eurasian perch vary widely in their DHA content, both within and between habitats. Pelagic perch consume prey with much higher DHA content (e.g. copepods and fish), whereas littoral perch consume large amounts of DHA-poor macroinvertebrates. B) Tree Swallows forage on insects originating from both aquatic and terrestrial habitats, but aquatic prey tend to have higher EPA. Some EPA-poor taxa like Hymenoptera and Thysanoptera are readily available in the environment whereas other high-EPA taxa like Ephemeroptera, Trichoptera, and Odonata are scarce (bars denote habitat availability). All of the rare aquatic prey are preferentially selected by Tree Swallows, relative to the more EPA-poor (points reflect variation in dietary proportions). Perch prey and diet data are from Chaguaceda et al. (2020) and Tree Swallow prey and diet data are from McCarty and Winkler (1999) and Twining et al. (2018).

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Fig. 4: A simplified fatty acid network including the following modules: (A) *de novo* synthesis of saturated fatty acids (SFA) via fatty acid synthase and a SFA elongase (B) conversion of SFA to monounsaturated fatty acids (MUFA) via [?]9 stearoyl-coA desaturase, (C) conversion of the MUFA oleic acid (18:1n-9, OA) to the n-6 PUFA linoleic acid (18:2n-6, LIN) via [?]12 methyl-end desaturase, (D) conversion of omega-6 PUFAs like LIN and arachidonic acid (20:4n-6, ARA) to the omega-3 PUFAs ALA (18:3n-3) and to EPA (20:5n-3) via the [?]15 and [?]17 omega desaturases respectively, (E) conversion of LIN to n-6 LC-PUFAs by front-end desaturases and PUFA elongases (F) conversion of ALA to n-3 LC-PUFAs by front-end desaturases and PUFA elongases. Note that Beta-oxidation process (multi-enzyme reaction) is also required to synthesise some LC-PUFA including DHA.

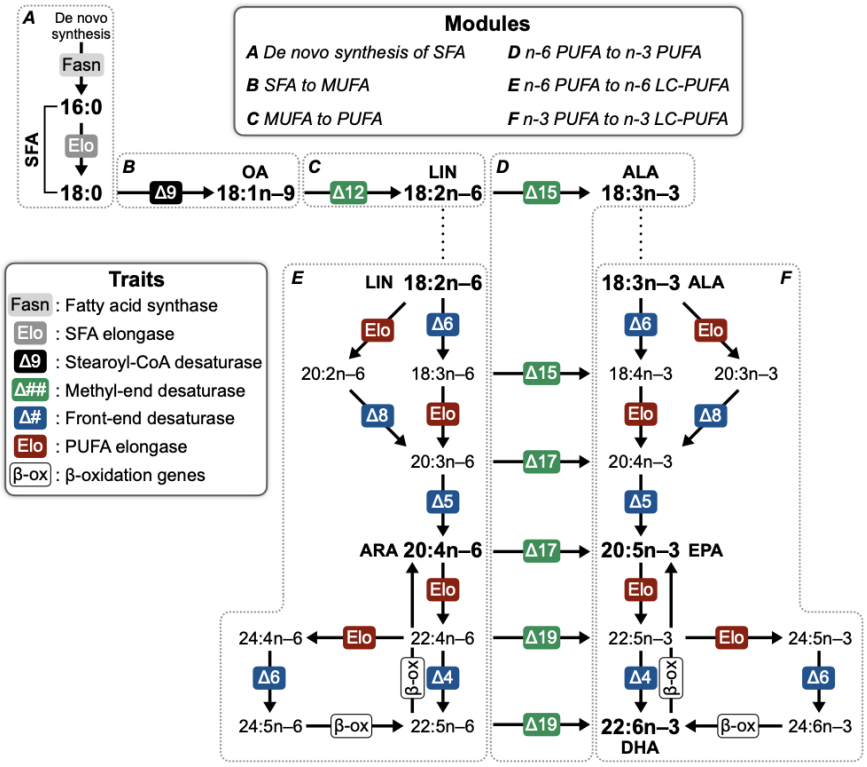


Fig. 5: Hierarchical structure of inter-related functional traits involved in fatty acids. Both shifts in sub-ordinate traits (metabolic traits; red box) and resource acquisition traits (behavioural, morphological and life-history traits; blue boxes) enable an organism to alter lipid traits and physiological traits (purple boxes), which finally influence individual fitness.

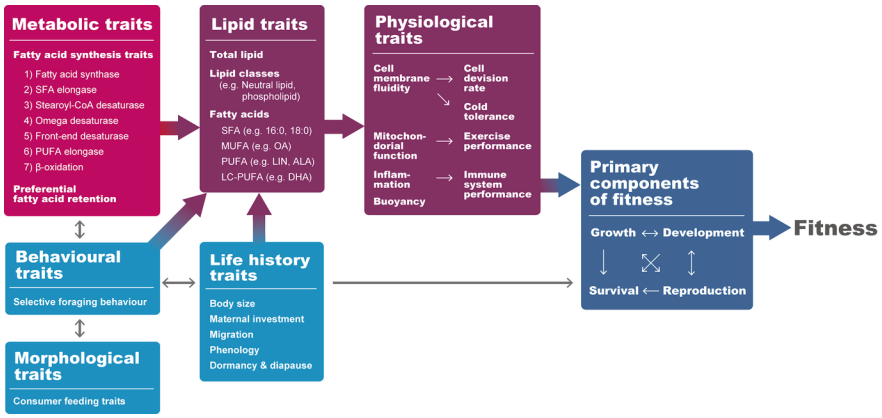
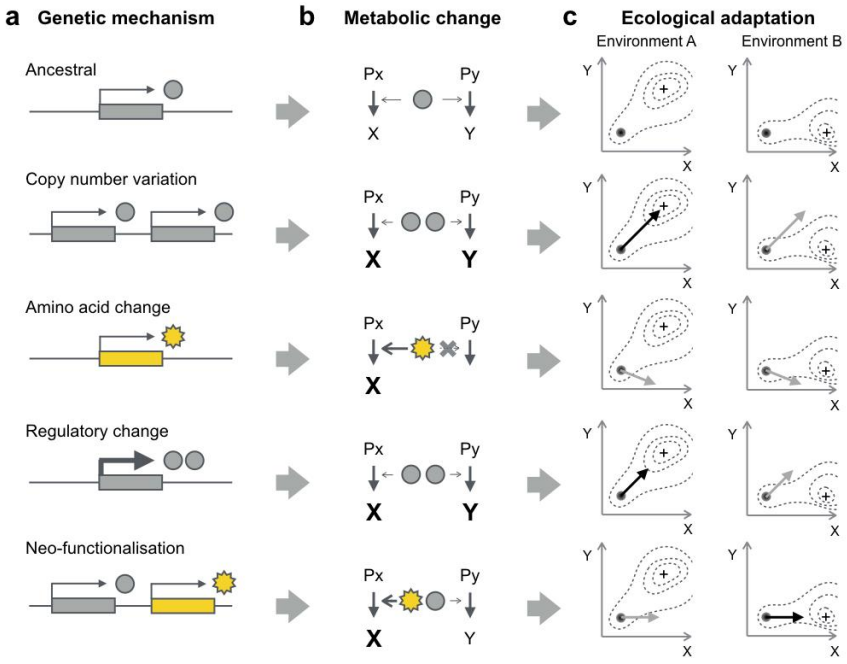


Fig. 6: Relationship between genetics and ecology illustrated on fatty acid adaptive landscapes. **a,** Different genetic mechanisms underlying increases in metabolic enzymatic activities. The arrow thickness indicates the gene expression levels. Gray circles and yellow asterisks indicate enzymes with ancestral and new functions, respectively. **b,** Effects of mutations on the metabolic pathways. Because of a pleiotropic effect of the enzyme, both metabolic pathways can be influenced. Font sizes reflect the amounts of synthesized fatty acids X and Y. Px, precursor of fatty acid X; Py, precursor of fatty acid Y. **c,** Adaptive (black) or non-adaptive walks (gray) on two different types of adaptive landscapes. The X and Y axes indicate the levels of fatty acids X and Y. Plus (+) indicates the adaptive peak. In Environment A, simultaneous increases of fatty acids X and Y are favored. In Environment B, however, only an increase in fatty acid X but not fatty acid Y is favored.



Box 1: Why the genetics of adaptation matters for the evolutionary ecology of fatty acids.

Quantitative genetic view of adaptation - Quantitative genetics models generally assume that traits are continuous, normally distributed, and controlled by many genes with small effects. In a model with a single trait, selection moves the trait distribution according to the breeder's equation: Response to selection (R)

is a product of heritability (h 2) and selection differential (S) (Lynch and Walsh 1998). Any heritable traits can evolve in response to selection. In cases in which multiple traits are genetically correlated, selection on one trait can bias the evolution of another genetically correlated trait, and the evolutionary trajectories on adaptive landscapes can be biased by a genetic variance-covariance matrix (G-matrix; Lande and Arnold 1983; Schluter 1996; Schluter 2000). Although quantitative genetics models are helpful to predict short-term evolution of highly polygenic traits, genomic studies of fatty acid composition have often identified loci with moderate to large effects (Cesar et al. 2014; Xia et al. 2014; Lemos et al. 2016; Lin et al. 2018; Horn et al. 2020), suggesting that an alternative view of the genetic basis of adaptation may be more useful in some cases.

Genomic view of adaptation - Recent advances in genomic technologies have improved our ability to elucidate the genetic details of adaptation. For instance, studies have helped document how aspects of genetic architecture, such as the number, effect sizes, pleiotropy, linkage, and genomic location of adaptive loci, can influence the speed and reversibility of adaptive evolution (Barton and Keightley 2002). Such studies also aim to identify causative genes. For instance, animal breeding studies have sought to identify genes and quantitative trait loci that control the fatty acid composition of meat (e.g., Kelly et al. 2014; Zhang et al. 2016). However, even when a locus with a major effect is identified, this does not necessarily indicate the presence of a single causative gene. Furthermore, even when a causative gene is identified, it does not necessarily mean that a single mutation causes the alteration of the gene function (Stern and Frankel 2013; Bickel et al. 2011). Therefore, an additional goal of genetic adaptation studies is to identify specific causative mutations (Lee et al., 2014; Remington, 2015). For example, *Fads2* duplication in freshwater species derived from marine ancestors allows them to synthesize more n-3 LC-PUFA (Ishikawa et al. 2019). When adaptation occurs via standing genetic variation, it may not be necessary to further dissect it into the levels of individual mutations for predicting how adaptation proceeds. This is because such adaptation occurs by replacement of already-existing alleles with tightly linked adaptive mutations (Barrett and Schluter 2008). However, to understand adaptation by *de novo*mutations, it is essential to determine the number and nature of responsible causative mutations (Stern and Frankel 2013). For example, the *bab* locus explains over 60% of phenotypic variance of pigmentation in *Drosophila* , but each single SNP explains only 1% (Bickel et al., 2011). Similarly, different *Fads1* and *Fads2* gene variants in humans explain between 1-28.5% of the variation in the PUFA content of blood phospholipids (Schaeffer et al. 2006). Once causative mutations that alter fatty acid synthesis are identified, it is then possible to determine whether adaptive evolution has occurred through a few large steps or multiple small steps (Orr 2005).

Box 1 Figure: Relationship between the enzymatic activity and fitness in two different environments (left) and a trajectory of adaptive walk (gray arrow) biased by G-matrix on a two-dimensional adaptive landscape (right). Px, precursor of fatty acid X; Py, precursor of fatty acid Y; Plus (+), the adaptive peak.

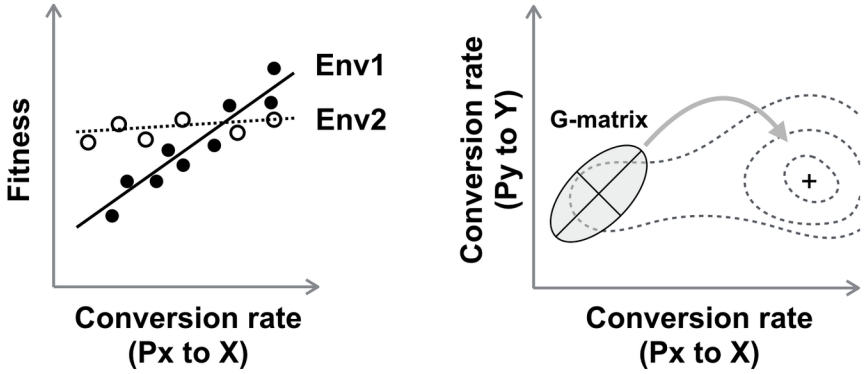


Table 1. Metabolic traits constituting the biosynthetic pathways of fatty acids

Pathway trait	**Trait type**	**Mechanism**	**Gene family**	**Example taxa with functional pathway**	**Example taxa without functional pathway**
1) 18:0	0/1	*De novo* fatty acid synthesis	Fatty acid synthase	All eukaryotes	
		Elongation	SFA elongases	All eukaryotes	
2) 18:0 18:1n-9 (SFA to MUFA)	0/1	[?]9 desaturation	First desaturases (Stearoyl-CoA desaturases)	All eukaryotes	
3) 18:1n-9 18:2n-6 (MUFA to PUFA)	0/1	[?]12 desaturation	Omega desaturases (Methyl-end desaturases)	Plants, protists, fungi, certain invertebrates (Cnidarians, Nematodes, Lophotrochozoans, some Copepods)	Deuterostomes
4) Omega-6 Omega-3	0/1	[?]15 desaturation (18:2n-6 18:3n-3)	Omega desaturases (Methyl-end desaturases)	Plants, protists, fungi, certain invertebrates (Cnidarians, Nematodes, Lophotrochozoans, some Copepods)	Deuterostomes
	0/1	[?]17 desaturation (C20 n-6 C20 n-3)	Omega desaturases (Methyl-end desaturases)		
	0/1	[?]19 desaturation (C22 n-6 C22 n-3)	Omega desaturases (Methyl-end desaturases)		
5) ALA EPA and LIN ARA	0/1	[?]6 desaturation	Front-end desaturases	Most vertebrates (*Fads*2 gene), invertebrates	Vascular plants (some can produce 18:4n-3) Most marine teleosts except Elopomorpha (lack *Fads*1)

Pathway trait	Trait type	Mechanism	Gene family	Example taxa with functional pathway	Example taxa without functional pathway
	0/1	[?]5 desaturation	Front-end desaturases	Most vertebrates (*Fads*1 gene); Fish with neofunctional-ization of *Fads*2 (e.g., Zebrafish, Salmon); invertebrates (e.g., nematodes, molluscs, sea urchins)	
	0/1	[?]8 desaturation	Front-end desaturases	Mammals, fish (*Fads*2 gene), invertebrates (e.g., molluscs, sea urchins)	
	0/1	Elongation (C18 C20)	PUFA elongases	Most animals	
6) EPA DHA and ARA 22:5n-6	0/1	[?]4 desaturation	Front-end desaturases	Microalgae, protists, fungi, fish with neo-functionalization of *Fads2* (e.g., Rabbit fish)	Vascular plants (no PUFA elongase) Some acanthoptery-gian fish (lack [?]6 desaturation to C24)
	0/1	[?]6 desaturation	Front-end desaturases	Mammals, fish (*Fads*2 gene)	
	0/1	Elongation (C20 C24)	PUFA elongases	Microalgae, protists, fungi, most animals	

Table 2. Types of intraspecific fatty acid traits that occur along alternative pathways to influence individual fitness components (Fig. 5). Full references are listed in our supplementary materials.

Pathway to optimal organismal omega-3 fatty acid content	Type of functional trait	Examples	Reference(s)
	SUBORDINATE TRAITS	**SUBORDINATE TRAITS**	**SUBORDINATE TRAITS**

Pathway 1: individual-level intracellular fatty acid conversion and regulation	**Pathway 1: individual-level intracellular fatty acid conversion and regulation** **Fatty acid metabolic traits** (Fig. 4) T1: fatty acid synthase T2: SFA elongase T3: stearoyl-CoA desaturase T4: omega desaturase T5: Front-end desaturase T6: PUFA elongase T7: Beta-oxidation genes	**Pathway 1: individual-level intracellular fatty acid conversion and regulation**	**Pathway 1: individual-level intracellular fatty acid conversion and regulation** Castro et al. 2012 Castro et al. 2016 Kabeya et al. 2018 Boyen et al. 2020
Pathway 2: individual-level organismal resource acquisition and life history	**Pathway 2: individual-level organismal resource acquisition and life history** **BEHAVIOURAL ACQUISITION TRAITS** Selective Foraging Behaviour	**Pathway 2: individual-level organismal resource acquisition and life history** **BEHAVIOURAL ACQUISITION TRAITS** Between prey items within an ecosystem: Calanoid copepods Rainbow trout (*Oncorhynchus mykiss*) Between seasons within an ecosystem European whitefish (*Coregonus lavaretus*) Tuatara (*Sphenodon punctatus*) Between ecotypes within an ecosystem: Killer whales (*Orcinus orca*) Threespine stickleback (*Gasterosteus aculeatus*) Between ecosystems: Eastern Phoebes (*Sayornis phoebe*)	**Pathway 2: individual-level organismal resource acquisition and life history** **BEHAVIOURAL ACQUISITION TRAITS** Eglite et al. 2019 Roy et al. 2020 Keva et al. 2019 Cartland-Shaw et al. 1998 Herman et al. 2005 Hudson et al. pers. comm.; Daneau-Lamoureux pers. comm. Twining et al. 2019
	Consumer Trophic Position	Ontogeny in Eurasian perch (*Perca fluviatilis*) Dietary plasticity in larval anurans	Chaguaceda et al. 2020 Whiles et al. 2010
	LIFE HISTORY TRAITS	**LIFE HISTORY TRAITS**	**LIFE HISTORY TRAITS**

	Body size	Freshwater calanoid copepods Round gobies and (*Neogobius melanostomus*) Monkey gobies (*Neogobius fluviatilis*)	Charette and Derry 2016 Ghomi et al. 2014
	Maternal investment Egg Composition (maternal effects) Milk Composition	Daphnia egg composition Tropical nudibranch egg composition (*Aeolidilla stephanieae*) Mammalian milk composition	Schlotz et al. 2013 Leal et al. 2012; Leal et al. 2013 Brenna et al. 2009; Hibbeln et al. 2019; Muhlhausler et al. 2011
	Migration	Migratory songbirds Migratory bats Southern hemisphere humpback whales (*Megaptera novaeanglidae*)	Pierce and McWilliams 2005; Pierce and McWilliams 2014 McGuire et al. 2013 Waugh et al. 2012
	Phenology	Colorado potato beetles (*Leptinotarsa decemlineata*)	Clements et al. 2019
	Hibernation	Black bears (*Ursus americanus*) Yellow-bellied marmots (*Marmota flaviventris*)	Iverson and Oftedal 1992 Hill and Florant 1999
	Dormancy & diapause	Harpacticoid copepods (*Heteropsyllus nunni*) Lepidopterans	Williams and Biesiot 2004 Vukašinović et al. 2015; Hemmati et al. 2017
	Morphology (consumer feeding traits)	Three-spine stickleback	Hudson et al pers. comm.; Daneau-Lamoureux et al. pers. comm.
Influenced by both Pathways 1 and 2	**Influenced by both Pathways 1 and 2**	**Influenced by both Pathways 1 and 2**	**Influenced by both Pathways 1 and 2**
	Lipid traits	**Lipid traits**	**Lipid traits**
	Total lipids	Fairy shrimp (*Chirocephalus diaphanus*) Euphausiids (*Meganyctiphanes norvegica*) Calanoid copepods (*Diaptomus kenai*)	Bocca et al. 1998 Mayzaud et al. 1999 Butler 1994
	Lipid classes (e.g., TAGs, polar lipids)	Euphausiids	Saito et al. 2002 Mayzaud et al. 1999

	Fatty acid content: SFA (e.g., 16.0, 18.0) MUFA (e.g., OA) PUFAs (e.g., LA, ALA) LC-PUFA (e.g., DHA)	Daphnia EPA content Copepod DHA content Sex-specific differences: Daphnia Tuatara Southern humpback whales	Hessen and Leu 2006; Wacker and Martin-Creuzburg 2007; Sperfeld and Wacker 2012 Charette and Derry 2016 Martin-Creuzburg et al. 2018 Cartland-Shaw et al. 1998 Waugh et al. 2012
	Body condition indexes -weight to length -fat mass	Tree Swallows (*Tachycineta bicolor*) Humans (*Homo sapiens*) Round gobies and Monkey gobies	Twining et al. 2016 Tan 2014; Elias and Innis 2001 Ghomi et al. 2014
	Physiological traits		
	Cell membrane fluidity	Eastern newt (*Notophthalmus viridescens*) Humans and Roundworms (*Caenorhabditis elegans*)	Mineo et al. 2019 Ruiz et al. 2019
	Mitochondrial function	Ground squirrels Red-winged Blackbirds (*Agelaius phoeniceus*) Humans	Gerson et al. 2008 Price et al. 2018 Herbst et al. 2014
	Inflammation	Immune system performance in Wolf spiders Daphnia Largemouth bass (*Micropterus salmoides*)	Fritz et al 2017 Schlotz et al 2012 Schlotz et al 2013 Schlotz et al 2016 Zhou et al 2020
	Buoyancy	Calanoid copepods (*Calanoides acutus*)	Pond and Tarling 2011
	Metabolic Rate and Exercise Performance	Thirteen-lined Ground Squirrels (*Spermophilus tridecemlineatus*) White-throated Sparrows (*Zonotrichia albicollis*) Yellow-rumped Warblers (*Setophaga coronata*) Atlantic salmon (*Salmo salar*) Mammals	Gerson et al. 2008 Price and Guglielmo 2009 Dick and Guglielmo 2019 McKenzie et al 1998 Ruf et al 2006
Pathway Products: Individual-level fitness component traits	**Pathway Products: Individual-level fitness component traits**	**Pathway Products: Individual-level fitness component traits**	**Pathway Products: Individual-level fitness component traits**

	Growth	Daphnia Tree Swallows Eastern Phoebes Freshwater fish	Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000; Ilić et al. 2019 Twining et al. 2016 Twining et al. 2019 Glencross 2009; Zhou et al. 2019 Lundova et al. 2018
	Development	Mammalian brain development Avian embryonic development	McNamara and Arsch 2019 Pappas et al. 2007
	Reproduction	Freshwater calanoid copepod fecundity Daphnia fecundity Eurasian perch gonad size	Charette and Derry 2016 Sperfeld and Wacker 2012; Ilić et al. 2019 Scharnweber and Gårdmark 2020
	Survival	Tree Swallow fledge success Sterlet survival (*Acipenser ruthenus*)	Twining et al. 2018 Lundova et al. 2018