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COMMENTARY

CENTENARY ARTICLE

What do molecular laws of life mean for species: absolute restrictions or mere suggestions?

Paweł Brzęk

ABSTRACT

Evolutionary biologists are interested in finding universal patterns of covariation between macroscopic and molecular traits. Knowledge of such laws of life can be essential for understanding the course of evolutionary processes. Molecular parameters are presumably close to fundamental limits set to all organisms by laws of physics and chemistry. Thus, laws of life that include such parameters are hypothesized to be similar at both wide interspecific levels of variation and narrower levels of intraspecific and intraindividual variation in different species. In this Commentary, I discuss examples where the significance or direction of such molecular laws of life can be compared at different levels of biological variation: (1) the membrane pacemaker theory of metabolism, (2) the correlation between variation in metabolic rate and mitochondrial efficiency and (3) the allometric scaling of metabolism. All three examples reveal that covariations within species or individuals that include molecular parameters do not always follow patterns observed between species. I conclude that limits set by molecular laws of life can be circumvented (at least to some degree) by changes in other traits, and thus, they usually do not impose strict limitations on minor within-species evolutionary changes (i.e. microevolution). I also briefly discuss some of the most promising perspectives for future studies on the universality of molecular laws of life.

KEY WORDS: Molecular parameters, Laws of life, Natural variation, Membrane pacemaker theory of metabolism, Mitochondrial efficiency, Metabolic scaling

Introduction

In recent decades, the research activity of evolutionary biologists has gradually broadened from typical 'macroscopic' traits such as body mass, litter size or whole-body metabolic rate to include molecular parameters (hereafter, my definition of 'molecular parameters' does not include genetics). Although this trend does not represent a real novelty (see Hochachka and Somero, 1973), many scientific meetings may currently leave participants with an impression of almost a 'fusion' between evolutionary and molecular biology. This change is driven by a tendency to look for mechanisms responsible for observed evolutionary processes, and benefits from both technical progress that has made sophisticated biochemical and biophysical methods more feasible, as well as growing cooperation between evolutionary and molecular biologists. The widespread use of molecular methods has added a new dimension to the way in which evolutionary biologists see objects of their studies. We are now aware of tremendous variation that is present in nature at the molecular level and we know that it can underlie variation in macroscopic traits (Konarzewski and Książek, 2013; Kozłowski et al., 2020; Koch et al., 2021).

The fundamental assumption of evolutionary biology is that individual traits of organisms cannot change independently of other traits; furthermore, variation in one trait can be related to variation in the other traits because of pleiotropic effects, genetic correlations or trade-offs (see Glossary; Flatt and Heyland, 2011; Mauro and Ghalambor, 2020; Garland et al., 2022). Knowledge of how different traits are integrated is the key issue for understanding how organisms can respond to either natural selection (Armbruster et al., 2014) or human-induced challenges. Evolutionary biologists are especially interested in finding patterns of covariation between traits that are common for different groups of organisms. This tendency is understandable since the widespread observation of these patterns suggests that there is a 'law of life' shaping the course of evolution that we can detect. Such a tendency is justified because: (1) all organisms studied by evolutionary biologists are (closely or more distantly) related to each other; (2) many molecular pathways are remarkably conserved during evolution (Khan et al., 2019; Husak and Lailvaux, 2022); and (3) the laws of physics and chemistry are common to all organisms.

What can we expect about the generality of laws of life that involve molecular parameters? Such parameters are presumably closer to the fundamental limits set for all biological processes by chemical and physical laws than macroscopic traits. Genetic correlations or developmental coupling between traits can limit the rate of evolution but not completely preclude it (e.g. Beldade et al., 2002). Conversely, even the strongest pressure of natural selection cannot overcome molecular laws of life if they represent physical and chemical constraints. Therefore, covariation among traits that involve molecular parameters should be hypothesized to be similar in different groups of organisms, as well as to be found at different levels of cladistic hierarchy; such molecular rules could be as strict as genetic code and could be broken only by a new set of molecular parameters. For example, minimum and maximum metabolic rates are correlated in most vertebrate groups but not in the case of cold-induced metabolism in mammals, presumably because of the molecular background of thermogenesis in this group (Auer et al., 2017).

On wide and narrow variation in molecular parameters

The integration of biological processes at different scales is a key challenge in modern biology (St Mary et al., 2021). Although biological variation occurs along the whole gradient of such 'scales', I will focus on two distinct levels, which I define as 'wide' and 'narrow' variation. Wide variation represents the level that is usually called interspecific variation and refers to variation within large groups such as ectotherms, birds and placental mammals. Narrow variation represents intraspecific variation, which encompasses differences between individuals, populations or lines

Faculty of Biology, University of Białystok, Ciołkowskiego 1J, 15-245 Białystok, Poland.

*Author for correspondence (brzek@uwb.edu.pl)

P.B., 0000-0003-4524-3834

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Glossary

Allometric scaling
When a value of a particular trait in different organisms does not change proportionally to their size (such a relationship becomes linear only when the value of a trait and body size are log-transformed).

Basal metabolic rate
Metabolic rate measured in an endotherm at thermoneutrality, in animal that is resting, postabsorptive, non-growing and not reproducing. As such, it is measured only in endotherms.

DHA
Docosahexaenoic fatty acid (22:6), a highly unsaturated fatty acid that can play an important role in determining properties of cell membranes.

Genetic correlation
The proportion of variation in two traits that is shared because of genetic causes.

Pleiotropic effect
When the same gene affects two or more traits.

Resting metabolic rate
The metabolic rate measured in an endotherm that is resting. This is sometimes used in situations when strict requirements of measurements of basal metabolic rate cannot be fulfilled.

Trade-off
When an increase in fitness through change in one trait results in a detrimental change in another trait.

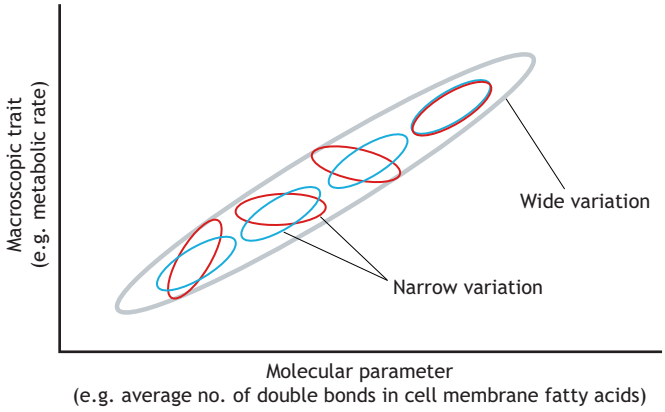

Fig. 1. An illustration of two possible patterns of correlation between molecular parameters and macroscopic traits at the level of wide and narrow variation. The example depicted here is the membrane pacemaker theory of metabolism. The direction of correlation between molecular parameters and macroscopic traits can be the same at wide and narrow levels of variation (blue ovals). Alternatively, the correlation between molecular parameters and macroscopic traits at the narrow level of variation may not always be the same as the pattern observed at the wide level of variation (red ovals).

within the same species, and intraindividual variation, i.e. phenotypic flexibility. Thus, narrow variation in molecular parameters can (at least theoretically) directly affect individual fitness and microevolutionary changes. It is also important to note that the relative magnitude of both wide and narrow variation can vary among different groups and species; nevertheless, the range of wide variation in a particular group should be always larger than the range of narrow variation in each species belonging to this group.

Molecular laws of life are usually described at the level of wide variation simply because such variation guarantees much higher (and thus more evident/detectable) differences between studied species. Such studies usually try to control and reduce the impact of narrow variation on analysed parameters, for example, by assaying all individuals at the same age and at the same time of day or simply averaging all measurements from a large sample size. However, the question of whether the same pattern of covariation between traits can be found at the level of narrow variation (Fig. 1) is important for several reasons. First, natural selection acts on individuals of the same species, so any truly strict law of life should also be obeyed at this level. Testing molecular laws of life at the intraspecific level can also avoid the need for correction of compared variables for phylogeny that is usually necessary in comparisons based on wide variation. The narrow variation is also relevant for tests of whether particular laws can limit the performance of individuals and their capacity to deal with environmental or climatic changes, for example. Thus, the uniformity of molecular laws of life – or the lack thereof – is directly related to the more general problem of macrophysiology, that is, variations in physiological traits over large geographical and temporal scales (Chown et al., 2004; Chown, 2023). Finally, such uniformity may have direct, practical benefits for scientists; if a simple molecular parameter quantified in a small sample represents a reliable index of a complex parameter in a wide array of individuals, it could allow for more feasible and sometimes also more ethical studies; for example, in finding potential links between molecular factors in the blood and whole-body metabolic rates (Malkoc et al., 2021; Holden et al., 2022).

The lack of significant covariation between molecular and macroscopic traits at a narrow level of variation can simply reflect the lower power of such tests (as this variation is usually smaller than wide variation). However, more interesting are the situations when we can find significant variation in one trait without simultaneous variation in its presumed molecular correlate [e.g. changes in basal metabolic rate (BMR, see Glossary), without changes in mitochondrial efficiency; Brzęk et al., 2022] or when the direction of the link between two traits found at the narrow level contradicts the pattern observed at the wider scale (e.g. correlation between basal metabolic rate and the composition of cell membranes; Brzęk et al., 2007; see Fig. 1). I emphasize that such situations do not necessarily mean that the molecular law of life is falsified. Rather, they suggest that a particular law of life represents some general constraint set by physics and chemistry on large-scale evolutionary changes; however, simultaneously, such a law allows for some freedom of action for natural selection that does not necessarily need to obey it during its daily work. For example, instead of changes in molecular parameters affecting mass-specific metabolic rate, evolution can simply change the mass of internal organs.

Below, I discuss several cases demonstrating that the relationship between wide and narrow variation is not always straightforward, and how these examples relate to the molecular laws of life and evolutionary physiology.

Do cell membrane fatty acids regulate the metabolic rate?

A classic example linking the variation in relatively simple molecular parameters to an important macroscopic trait is the membrane pacemaker theory of metabolism (hereafter referred to as ‘the membrane pacemaker theory’) proposed by Hulbert and Else (Hulbert and Else, 1999), which is explained in detail elsewhere (Hulbert and Else, 2000; Hulbert, 2007). This theory is based on two observations: (1) molecular processes associated with cell membranes represent an important component of the ‘whole body’ metabolic rate (particularly BMR in endotherms) and (2) properties of cell membranes depend on the composition of membrane phospholipids, particularly the abundance of polyunsaturated fatty acids. Thus, it was proposed that the composition of cell membrane

fatty acids can modulate the rate of metabolic processes. Moreover, since double covalent bonds are more sensitive to oxidative stress than single bonds, the variation in the saturation of cell membrane fatty acids can also explain variation in lifespan (Hulbert et al., 2007; Hulbert, 2008, 2010).

The membrane pacemaker theory offers a simple, mechanistic explanation for variation in metabolic rate; it is easy to visualize that cell membranes with more 'bent' unsaturated fatty acid chains are more fluid-like, allowing for increased dynamics of large enzyme molecules or higher levels of proton leakage. The membrane pacemaker theory is supported by interspecific comparisons, revealing that the average number of double covalent bonds in cell membrane fatty acids correlates negatively with body mass and positively with the activity of key metabolic enzymes (e.g. Hulbert et al., 2002a,b; Brand et al., 2003; Turner et al., 2003, 2005).

Several authors have tried to test the validity of the membrane pacemaker theory at a narrower range of variation in metabolic rate. The most common approach has been to compare the composition of cell membranes in individuals with different metabolic rates, taking advantage of either natural variation (Haggerty et al., 2008; Sukhotin et al., 2017) or artificial selection (Brzęk et al., 2007; Wone et al., 2013; Stawski et al., 2015). None of those studies unequivocally support the membrane pacemaker theory. Other authors have analysed the effect of diet-induced manipulation of fatty acyl composition on metabolic rate, although the results of such experiments are perhaps more ambiguous, as the level of unsaturation of cell membranes fatty acids can be regulated homeostatically in spite of diet variation (Hulbert et al., 2014). However, even when dietary manipulation is strong enough to affect behavior (Pannorfi et al., 2012) or directly modulate the composition of cell membrane fatty acids (Price et al., 2018), it still does not change the metabolic rate. There is better support for the presence of an intraspecific link between cell membrane fatty acyl composition and longevity, usually based on studies comparing strains with different lifespans (Hulbert et al., 2006; Shmookler Reis et al., 2011; Valencak and Ruf, 2013 but see Moghadam et al., 2013). However, no correlation was found between longevity and fatty acyl composition in a between-population comparison of a potentially extremely long-living bivalve, *Arctica islandica* (which can live up to 507 years; Rodríguez et al., 2019). This result is particularly remarkable, as such a link was observed in a study comparing *A. islandica* with other bivalves (Munro and Blier, 2012), and the range of narrow and wide variation in lifespan in both studies was comparable. Finally, it has been noted that the validity of the membrane pacemaker theory, even at the interspecific level, can be affected by confounding effects of phylogeny and body mass (Calhoon et al., 2015). For example, the link between the unsaturation level of cell membrane fatty acids and BMR in mammals is not significant when compared variables are controlled for the effects of phylogeny and body mass (Valencak and Ruf, 2007). A similar interspecific study in birds provided only very weak support for the membrane pacemaker theory (Kumar et al., 2021).

Taking all these observations into account, it is not surprising that even the author of the membrane pacemaker theory has admitted that intraspecific variation in metabolic rate is not necessarily related to membrane fatty acid composition (Hulbert, 2010). Undoubtedly, the number of double bonds in fatty acyl chains can modulate the properties of the cell membrane and affect the rate of metabolic processes, and shrews cannot achieve their metabolic rate if they have the same composition of liver fatty acids as elephants. However, organisms are obviously able to adjust their metabolic rate to some extent without following the predictions of the membrane pacemaker theory. It can be done in several ways, such as by changing organ size (Brzęk et al., 2007) or modulating parameters of cell membranes other than the unsaturation index (Calhoon et al., 2015).

It is also important to note that the membrane pacemaker theory was originally proposed specifically for BMR (Hulbert and Else, 1999). However, it has been observed that hypoxia-induced reduction in the resting metabolic rate (RMR, see Glossary) in naked mole-rats is accompanied by a decrease in fatty acyl unsaturation in the liver and an increase in muscle (Farhat et al., 2020). This can either confirm Hulbert and Else's theory at an intraindividual level or contradict it, depending on which organ is assumed to be the better predictor of the RMR. Moreover, natural selection acting directly on the BMR in endotherms should invariably minimize its value to reduce maintenance costs, and the evolution of a higher BMR could occur only as a correlated response to selection acting on other fitness-related traits (Swanson et al., 2017). Because fatty acids fulfil different functions in organisms (Arnold et al., 2015; Harayama and Riezman, 2018), this mechanism can weaken the correlation between BMR and fatty acyl composition at the intraspecific level, since the observed composition of fatty acids can represent a compromise between the BMR (i.e. the predictions of the cell membrane theory) and the demands of other functions. Similarly, even at a wide interspecific level, the BMR can be correlated with mean unsaturation of cell membrane fatty acids but not with the relative abundance of almost all specific fatty acids (Kumar et al., 2021).

Finally, the composition of cell membrane fatty acids can affect the intraspecific variation in metabolic rate even if the mechanism of this action differs from that proposed by the cell membrane pacemaker theory. For example, divergent selection for the BMR in laboratory mice modulated liver fatty acyl unsaturation and the abundance of docosahexaenoic acid (DHA, see Glossary), but in a direction contradicting the cell membrane pacemaker theory (Brzęk et al., 2007). Nevertheless, the fact that selection acting on BMR modulated the composition of cell membrane fatty acids reveals the presence of significant links between them (see also Czajkowska et al., 2019). Interestingly, mice selected for high BMR grow faster (Sadowska et al., 2013) and have larger livers but with a lower abundance of DHA than their counterparts with a lower BMR (Brzęk et al., 2007). In red-winged blackbird nestlings, liver DHA content increases soon after hatching and can reach values higher than those observed in adult birds, followed by an increase in DHA in pectoral muscles during the later nestling period (Price et al., 2018). These patterns parallel changes in the relative size of respective organs in altricial birds (Lilja, 1982) and may be interpreted as an adaptation for rapid growth. Thus, the faster growth rate in both cases is related to a larger liver but not necessarily to more unsaturated liver cell membrane fatty acids. This is another example where the solutions of biological problems do not always need to follow the same laws.

What is the direction of correlation between mitochondrial efficiency and metabolic rate?

Since mitochondria are responsible for most energetic processes in most organisms, they can play a key role in the evolution of life histories (Koch et al., 2021). Here, I will focus only on variation in mitochondrial efficiency. We are now aware that mitochondrial ATP production during aerobic respiration is not coupled to units of consumed oxygen in a fixed ratio. Mitochondrial efficiency is a complex trait that reflects variation in several mitochondrial parameters, such as proton leak and the type of substrate used

(Salin et al., 2015) or properties of respiratory chain activity (Roussel et al., 2018). In general, organisms with more efficient mitochondria can obtain more energy from the same amount of food but are at risk of higher oxidative stress. Therefore, variation in mitochondrial parameters at the molecular level may have far-reaching evolutionary consequences for the whole organism (Salin et al., 2015; Koch et al., 2021; Metcalfe and Olsson, 2022).

The general (and perhaps not surprising) pattern that emerges from studies analysing variation in mitochondrial efficiency at a narrow level of variation is that more efficient mitochondria are linked to faster growth (Toyomizu et al., 2011), better locomotor capacity (Roussel et al., 2020) or higher parental investment (Park et al., 2020). Strikingly, although nestlings of altricial birds are less physiologically mature than adult birds (Starck and Ricklefs, 1998), younger chicks can have more efficient mitochondria than older nestlings (Cossin-Sevrin et al., 2022), presumably to improve their growth capacity. Conversely, a comparative analysis of wide, interspecific variation in mitochondrial efficiency in mammals found a negative correlation between BMR and mitochondrial efficiency (Boël et al., 2019). Thus, although higher mitochondrial efficiency is observed in individuals with higher rates of energy expenditure at the intraspecific and intraindividual levels in endotherms, it is negatively correlated with BMR at the interspecific level. These contrasting patterns are unexpected, as models explaining the evolution of endothermy frequently assume that BMR is correlated positively with the capacity of maintaining higher aerobic metabolic rate during locomotor activity or parental investment, for example (Bennett and Ruben, 1979; Koteja, 2000). Thus, one could expect that the correlation between variation in metabolic rate and variation in mitochondrial efficiency should always be the same.

There are several possible explanations for this apparent paradox. First, the negative correlation described by Boël et al. (2019) is most evident for basal metabolism but gradually becomes weaker for higher rates of ATP synthesis and becomes nonsignificant at its maximum rate. At the same time, mitochondrial efficiency improves when metabolic rate increases (Boël et al., 2019). Thus, the described contrast between wide and narrow variation in mitochondrial efficiency could be (at least) less pronounced when we consider the average rate of metabolism during effort (probably intermediate between basal and maximal). Moreover, the interspecific correlation between mitochondrial efficiency and BMR (Boël et al., 2019) has been hypothesized by these authors to reflect higher proton leakage in smaller species with less efficient mitochondria (and the intensity of proton leakage in turn can depend on the fatty acyl composition of cell membranes). However, the flexibility of mitochondrial efficiency can also depend on mechanisms other than proton leakage (Salin et al., 2015; Roussel et al., 2018). For instance, an increase in proton leakage does not preclude an increase in mitochondrial efficiency if it is offset by a simultaneous increase in ATP-synthesis capacity, reflecting changes in, for example, the mitochondrial electron transport system (Bryant et al., 2018). Finally, the evolution of the BMR in mammals at a narrow range of variation is possible without simultaneous changes in mitochondrial efficiency (Brzęk et al., 2022). Thus, there is no need to expect inter- and intraspecific variation in mitochondrial efficiency at rest and during activity to reflect the same proximate molecular mechanisms.

Scaling of metabolic rate: is there one general law of life?

It is almost impossible to write even a very concise text about evolutionary physiology without touching the problem of the allometric scaling (see Glossary) of metabolic rate. The deceivingly simple question of why larger organisms have lower mass-specific rates of metabolic processes than smaller ones has been the subject of innumerable studies (Glazier, 2014; White and Kearney, 2014; Kozłowski et al., 2020; Harrison et al., 2022). Essentially, this is the most famous example of the question about the generality of laws of life; allometric scaling is so prevalent that it seems to reflect the presence of one fundamental rule that affects the functioning of all living things. Indeed, several theories that try to explain allometric scaling are not even based on molecular parameters but rather on more fundamental physical constraints set by the surface-to-volume ratio (e.g. Kooijman, 2010) or limits of the branching transport networks (e.g. West et al., 1997; Banavar et al., 1999), for example. Such constraints should be particularly likely to apply in all groups; indeed, it has been emphasized that their implications can be observed across the whole phylogenetic tree of life (West and Brown, 2005; Brown et al., 2022). By contrast, many authors have noted that the shape of allometric relationships can vary between different groups (e.g. Bokma, 2004; Clarke et al., 2010) or can even evolve (e.g. Glazier et al., 2011), making doubtful the presence of one universal and inflexible rule. Finally, it has been proposed that both inter- and intraspecific metabolic allometries can arise not as a result of single physical constraint but rather emerge as a consequence of evolutionary optimization of life history parameters, such as the allocation of resources to growth and reproduction (Kozłowski et al., 2020; White et al., 2022).

This short summary reveals that there are two contrasting approaches to the problem of allometric scaling of metabolic rate: one emphasizes the universality of scaling patterns and the importance of physical constraints, another is more interested in biological variation in scaling patterns and the variety of factors that can affect them. Both approaches presumably represent two sides of the same coin. Strikingly, two recent reviews, written from either a more physical (Brown et al., 2022) or more biological (Harrison et al., 2022) point of view, agree that physics and geometry impose some general constraints on organisms, but natural selection can overcome them – at least at the scale of what I call narrow variation (personally, I think this conclusion applies to all molecular laws of life). Indeed, many authors emphasize natural selection offers a range of different solutions to the same problem arising in different organisms (Harrison, 2018; Kozłowski et al., 2020). Thus, studies of generality (or the lack thereof) of molecular laws of life at different levels should be particularly helpful for solving the mysteries of mechanisms behind the allometric scaling of metabolic rate. Moreover, previous studies of allometric scaling have usually, although not exclusively (Glazier, 2005; Czarnołęski et al., 2008; Glazier et al., 2020), investigated wide interspecific variation in metabolic rate. In fact, one of the suggestions recently proposed by Harrison et al. (2022) to advance studies of the mechanisms responsible for allometric scaling is to focus more on intraspecific variation.

Conclusions and opportunities

The main conclusion that can be drawn from the above examples is that molecular patterns observed at the level of wide variation do not need to be obeyed strictly at the level of narrower variation and that patterns observed for narrow variation in different groups can vary. Graphically, molecular laws of life form corridors that allow for some ‘sideways movements’, rather than a rope along which organisms can move only backwards and forwards (Fig. 1). Such sideways movements can occur both at the macroscopic level (e.g. changes in body size and organ mass) and at the molecular level

(e.g. changes in cell size). In other words, limits set by one law of life can be circumvented with the help of other laws of life. Interestingly, the link between cell size and mass-specific metabolic rate (theoretically a very rigid law of life) is more variable at the intraspecific level than at the interspecific level, presumably because at the intraspecific level it can be more easily obscured by other factors (Glazier, 2022). This is the same pattern as that described above for the membrane pacemaker theory.

It is difficult to predict the future of molecular approaches in evolutionary biology, as it depends on (unpredictable) technical progress. Nevertheless, some advice could be proposed. The most obvious suggestion is to investigate more thoroughly the molecular background of variation in macroscopic traits at both narrow and wide levels of variation. In this respect, particularly interesting species would be those with considerable narrow variation, such as clams that were alive during the 16th century (Munro and Blier, 2012), beetles with a 30-fold range of variation in body mass (Somjee et al., 2021) or unintended long-term artificial selection experiments, such as the domestication of dogs (Jimenez, 2016). Similarly, any species with large plasticity or ontogenetic changes would represent a good research model. Here, particularly interesting are young altricial birds that combine the fastest growth rate in the animal kingdom (Case, 1978) with significant changes in their mitochondrial coupling or DHA content. However, it would also be important to study species that are otherwise not particularly 'peculiar' but are 'scattered' along less investigated branches of phylogenetic trees to improve our knowledge of interspecific variation using comparative methods. Sometimes including species from new groups can affect the final conclusions. For example, Gonzalez et al. (2015) found a significant link between body mass and the abundance of DHA fatty acid within the family Cyprinidae, but this relationship disappeared when a few species from another family, Catostomidae, were added.

New research methods could offer new opportunities for evolutionary biologists; in particular, methods that allow for the better quantification of molecular parameters and processes either in preserved samples (e.g. Acin-Perez et al., 2020) or *in vivo*. The former could be very helpful for field studies, whereas *in vivo* assays are necessary for better understanding of how analysed processes are integrated. The introduction of new methods will also make it more essential that we at least try to standardize the way those methods are used. This should be particularly important for comparative studies at the wide level of variation; thus far, interspecific comparisons of molecular parameters usually rely on samples analysed in the same team/laboratory.

Finally, the question of the generality of molecular laws is also relevant for translational studies. The knowledge of any exception from general laws of life can potentially enhance our understanding of how diseases evolve and how they can be treated. For example, it is known that some groups of organisms evolved genetic and molecular mechanisms, resulting in lower cancer risk that could be expected based on comparison with other species (Tollis et al., 2019). Additionally, sophisticated research tools such as organoids or organs-on-a-chip that are developed specifically for biomedical studies in humans could one day also become available for evolutionary biologists; then, the knowledge of (dis)similarity of molecular laws of life in *Homo sapiens* and other species could be invaluable in tuning those new tools.

To summarize, molecular laws of life in biology may be strict at the very general level, but they leave room for so many exceptions that they will certainly guarantee many exciting papers during the second century of Journal of Experimental Biology.

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Competing interests
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