

of electrons or holes through the material; and the thermal conductivity, which defines how well the material transports or equilibrates heat. Semiconductors that have a reasonably large specific electrical conductivity (in the range of thousands of siemens per centimetre) and a passable Seebeck coefficient (hundreds of microvolts per kelvin) are ideal candidates for efficient thermoelectric power generation, but only if the thermal conductivity is small enough to retain the necessary temperature difference effectively.

Different strategies have been developed to optimize these properties. Doping has commonly been used to increase the concentration of mobile charge carriers and holes, or to manipulate the electronic structure of semiconductors. This strategy has worked well in the case of lead telluride, leading to the development of heavily doped substances such as $\text{PbTe}_{1-x}\text{Se}_x$ (ref. 2; Se is selenium), and to a class of thermoelectric³ materials known as lead antimony silver tellurides. The Seebeck coefficients of thermoelectric materials can also be improved by tailoring their electronic structures⁴.

Most efforts to improve the thermoelectric properties of materials, however, have involved the reduction of thermal conductivity. This requires sophisticated methods, such as altering the nanometre-scale structure of a bulk material (in some cases generating well-defined low-dimensional substructures, such as quantum dots and quantum wells), or forming precipitates of another substance within a thermoelectric material. These approaches prevent heat transport⁵ by scattering phonons — the heat carriers in thermoelectric materials. But at least some of these widely used scattering procedures will be hard to scale up for the manufacture of commercial products in the near future.

The brilliance of Biswas and co-workers' study of lead telluride is that they have canalized almost every known strategy for optimizing thermoelectric materials into one system. They used a fast, highly effective technique known as spark plasma sintering (SPS) to synthesize bulk lead telluride, identified strontium telluride as the most suitable candidate to form nanoscale precipitates during the synthesis, and determined the optimal amount of sodium to use as a dopant. This combined approach improves the thermoelectric performance of lead telluride to previously unattainable levels.

The success of the authors' strategy depends on the interplay and occurrence of units at several different length scales: from mesoscopic grains of lead telluride at the micrometre scale, to nanoscale precipitates of strontium telluride, and all the way down to dopants that act at the atomic scale (Fig. 1). The authors call this interplay a panoscopic approach, but the embedding of progressively smaller subunits within the material reminds me of matryoshka (Russian) dolls.

Materials scientists have long dreamt of a fast, reliable method for producing bulk thermoelectrics that does not require complicated optimization procedures and intensive material structuring. Biswas and colleagues' work certainly provides a practical method for making bulk lead telluride, but it also shows us that we really do need to screen for the best additives and dopants, and to tailor structural units, to realize the panoscopic approach for every thermoelectric system. It should also be noted that lead telluride is toxic — for commercial applications, other thermoelectric materials must be found that are non-toxic and inexpensive.

Nevertheless, I am sure that the authors' findings will trigger exponential progress in the performance of thermoelectric materials in general. Indeed, I believe that many surprising

and encouraging aspects of thermoelectric behaviour will be discovered as a result of their work. If so, then thermoelectric devices might eventually be improved until their efficiency becomes at least comparable to that of other state-of-the-art energy-conversion devices, such as those that convert solar or geothermal energy. ■

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1. Biswas, K. *et al.* *Nature* **489**, 414–418 (2012).
2. Pei, Y. *et al.* *Nature* **473**, 66–69 (2011).
3. Hsu, K. F. *et al.* *Science* **303**, 818–821 (2004).
4. Nilges, T. *et al.* *Nature Mater.* **8**, 101–108 (2009).
5. He, J. *et al.* *J. Am. Chem. Soc.* **132**, 8669–8675 (2010).

EVOLUTIONARY BIOLOGY

Insects converge on resistance

In a remarkable example of convergent evolution, insect species spanning 300 million years of divergence have evolved identical single-amino-acid substitutions that confer resistance to plant cardenolide toxins.

NOAH K. WHITEMAN & KAILEN A. MOONEY

Plants and herbivorous insects are the most diverse groups of multicellular organisms, and understanding this species profusion is a central problem in evolutionary biology. A key explanatory hypothesis¹ is that iterative co-evolution between plants that produce toxic compounds² (such as nicotine and mustard oils) and herbivorous insects that have resistance to these toxins³ drives the diversification of each group. Some toxins have an extremely broad mode of action, leading to the evolution of highly divergent detoxification mechanisms in the specialized herbivores that resist these compounds⁴. But other toxins have just one molecular target. The medically important cardiac glycosides (cardenolides), for example, block activity of animal $(\text{Na}^+\text{K}^+)\text{ATPase}$ ⁵, an enzyme that regulates ion gradients across the cell membrane. Writing in *Proceedings of the National Academy of Sciences*, Dobler *et al.*⁶ demonstrate how co-evolution between plants containing toxic cardenolides and the herbivorous insects that feed on them represents an exquisite case of convergent molecular evolution, in which distantly related insect species have evolved a common adaptive response in a single gene.

Cardenolides comprise a diverse group of triterpenoid-derived steroids and are produced

by at least 60 genera from 12 families of flowering plants, including *Asclepias* species, or milkweeds, and *Digitalis* species, or foxgloves. They disrupt the function of the $(\text{Na}^+\text{K}^+)\text{ATPase}$ by binding to the first extracellular loop of the enzyme's α -subunit. Dobler and colleagues studied the sequence of the gene encoding the $(\text{Na}^+\text{K}^+)\text{ATPase}$ in 18 cardenolide-resistant herbivorous insect species from 15 genera in 4 orders (Coleoptera, Lepidoptera, Diptera and Hemiptera). They found sequence changes leading to amino-acid substitutions in the α -subunit's first extracellular loop in 16 of the cardenolide-feeding species. The authors then used *in vitro* assays to show that cells expressing some of these altered sequences survive in the presence of the cardenolide ouabain, which is otherwise toxic to cultured cells.

Remarkably, one of the amino-acid substitutions (at position 122) is present in cardenolide-feeding species of all 4 orders, but not in any of 14 insect species (also from all 4 orders) that do not feed on cardenolide-producing plants. This suggests that resistance-conferring substitutions present in a subset of cardenolide-feeding species are the result of adaptive evolution that occurred repeatedly across 300 million years of insect evolutionary divergence. However, it is worth noting that some cardenolide-resistant species do not show adaptive changes in the

(Na⁺+K⁺)ATPase sequence, suggesting that there are probably other molecular routes to this common trait.

In addition to being a striking case of convergent molecular evolution, this adaptation to cardenolide-producing plants carries ecological implications that extend beyond plant–herbivore interactions (Fig. 1). Some cardenolide-resistant insects sequester these compounds, which provides protection against predators, and some also display warning coloration that advertises this defence⁷. Indeed, the majority of the cardenolide-feeding insects studied by Dobler *et al.* exhibit warning coloration and are highly toxic. These herbivore traits in turn have their own evolutionary impacts. Coexisting toxic species often evolve similar warning signals, presumably to spread the costs of ‘teaching’ predators avoidance — an evolutionary process described as Müllerian mimicry. And, in a process known as Batesian mimicry, palatable species sometimes evolve to mimic toxic ones, in order to usurp predator protection.

Although these phenomena — host plant specialization, sequestration of plant toxins, warning coloration, and mimicry — are quite common across herbivorous insects, cardenolide-feeding species have been central to the study of these dynamics. The work by Dobler *et al.* therefore adds a crucial molecular-genetic piece to this complex puzzle, establishing cardenolide-feeding herbivores as one of the best examples of how molecular, functional and ecological convergence can be linked.

However, fascinating questions remain. It is unclear whether the evolutionarily important mutations are fixed in each cardenolide-feeding species or whether intraspecific genetic variation exists. Comparing the ratio of non-synonymous changes (mutations that lead to an amino-acid substitution) and synonymous changes (mutations that do not change the amino acid) in the gene encoding the (Na⁺+K⁺)ATPase within and between species would help to reveal whether natural selection has fixed many other mutations — in addition to the functionally important ones already identified — in the cardenolide-feeding lineages⁸. Reconstructing the progression of all amino-acid substitutions in each species lineage could reveal whether interactions between mutations have played a part in the adaptive evolution occurring at this sequence⁹. Furthermore, there is variation in the abundance and structure of the cardenolides produced by plants, but we do not know whether this variation has been driven by population variation in insect resistance or vice versa.

It also remains to be confirmed that the amino-acid substitutions identified by Dobler and colleagues do actually confer

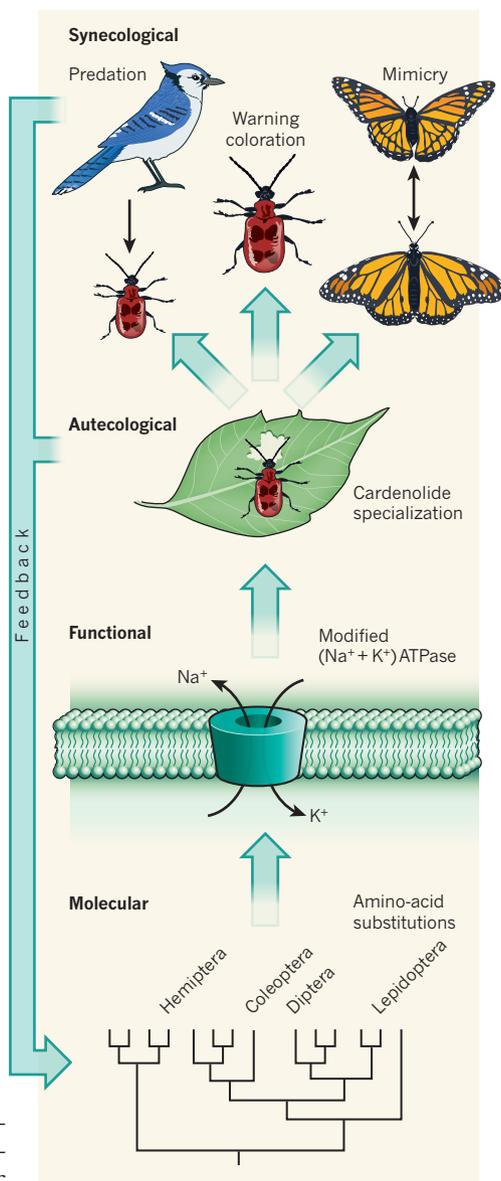


Figure 1 | Hierarchy of adaptation. Dobler *et al.*⁶ have identified a single amino-acid substitution in the gene encoding the enzyme (Na⁺+K⁺)ATPase in insect species belonging to four orders, which span 300 million years of evolutionary divergence. This (and other) substitutions cause modifications to the enzyme that confer resistance to toxic compounds called cardenolides, possibly allowing insects with this adaptation to feed on plants producing these toxins. This is an example of molecular convergence (the same genetic change provides the same functional outcome in multiple species) leading to autecological convergence (multiple species displaying the same ecological trait; in this case, the ability to feed on cardenolide-producing plants). Such processes can also have synecological effects — those that influence the ecology of other species. For example, some insect species sequester these same plant toxins to protect themselves from predator attack, and display warning coloration to advertise this. Coexisting insects can then evolve to mimic one another, adopting similar warning signals. These ecological effects can, in turn, feed back to influence molecular and functional convergence.

resistance to cardenolides in insects, because the authors tested the effects of these changes only *in vitro* in human cells. One approach to move beyond cell lines would be to engineer *Drosophila* fruitflies to express the resistance genes, and then conduct ouabain-feeding trials. Solving the crystal structure of just one (Na⁺+K⁺)ATPase from a cardenolide specialist would also allow for a more refined analysis of the molecular mechanisms underlying each substitution that confers resistance.

Finally, the findings of Dobler *et al.* pose additional ecological questions. For example, have similar convergent evolutionary mechanisms evolved in the predators that feed on cardenolide-laden insects? Black-headed grosbeak birds (*Pheucticus melanocephalus*) predate overwintering monarch butterflies (*Danaus plexippus*) in the Oyamel fir forests of Mexico¹⁰, but it is not known whether these birds, and other such predators, have also evolved cardenolide-resistant (Na⁺+K⁺)ATPases.

The patterns identified by Dobler and colleagues in the insect (Na⁺+K⁺)ATPase gene are likely to become a textbook example of convergent evolution at the molecular level — illuminating how a common selective agent can lead to a common set of evolutionary solutions. Their approach highlights the way in which modern evolutionary biology can pinpoint variations in genotype that lead to variation in molecular and organismal traits in ecologically relevant species, not just in model organisms¹¹. Ultimately, such studies are helping to identify the ecological forces and molecular mechanisms responsible for generating the diversity of life on Earth. ■

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- Ehrlich, P. R. & Raven, P. H. *Evolution* **18**, 586–603 (1965).
- Fraenkel, G. *Science* **129**, 1466–1470 (1959).
- Dethier, V. G. *Evolution* **8**, 33–54 (1954).
- Winde, I. & Wittstock, U. *Phytochemistry* **72**, 1566–1575 (2011).
- Rasmann, S. & Agrawal, A. A. *Ecol. Lett.* **14**, 476–483 (2011).
- Dobler, S., Dalla, S., Wagschal, V. & Agrawal, A. A. *Proc. Natl Acad. Sci. USA* **109**, 13040–13045 (2012).
- Bernays, E. A. *BioScience* **48**, 35–44 (1998).
- McDonald, J. H. & Kreitman, M. *Nature* **351**, 652–654 (1991).
- Aardema, M. L., Zhen, Y. & Andolfatto, P. *Mol. Ecol.* **21**, 340–349 (2012).
- Fink, L. S. & Brower, L. P. *Nature* **291**, 67–70 (1981).
- Dean, A. M. & Thornton, J. W. *Nature Rev. Genet.* **8**, 675–688 (2007).