Selection mediated by both pollinators and herbivores affects the evolution of floral traits.

tractiveness to pollinators, but at the same time increase the risk of damage from herbivores. Optimal floral display will then be affected by the relative strength of these effects and their consequences for plant fitness (*6*). Moreover, because damage from herbivores can reduce attractiveness to pollinators, interactions with herbivores may influence the degree of pollen limitation and selection on traits affecting the ability to produce offspring through autonomous self-pollination (*2*). In addition, evolutionary response will depend not only on the strength and direction of selection, but also on the presence of genetic variation and genetically based correlations among traits.

Experimental evolution is a powerful approach to examine how environmental conditions affect evolutionary trajectories. Ramos and Schiestl combined pollination and herbivory treatments to determine their independent and interactive effects on the evolution of floral traits. A rapid-cycling population of the annual plant *Brassica rapa* evolved under one of four treatment combinations: hand-pollination with or without herbivory, and bee pollination with or without herbivory. In two treatment combinations, pollination by hand ensured that flowers received a surplus of pollen, whereas in the other two, bumble bees determined the pollination success of individual plants. As predicted, selection by bumble bees resulted in the evolution of plant phenotypes that were more attractive to bumble bees. This effect was reduced when plants evolved in the presence of both bumble bees and leaf herbivores (caterpillars of the butterfly *Pieris brassicae*). The observations are consistent with the idea that plant traits that influence attractiveness to pollinators may be subject to conflicting selection from herbivores. In addition, plants pollinated by bumble bees, which were presumably more strongly pollen-limited than were plants pollinated by hand, tended to evolve greater ability to produce offspring through autonomous self-pollination.

More unexpectedly, the presence of herbivores was associated with an increased efficiency of autonomous self-pollination also among plants pollinated by hand. This cannot be explained by an effect of herbivores on pollen limitation. Instead, Ramos and Schiestl suggest that this is the result of genetic correlations between traits affecting resistance to herbivory and floral traits affecting efficiency of autonomous self-pollination. This hypothesis could be tested by artificial selection (selective breeding) on these traits.

The experimental treatments were applied for six generations, which was sufficient for selection lines to diverge in several traits. One reason is that the population of *B. rapa* used in the study is genetically highly variable. The population was established through crosses between early-flowering genotypes and many generations of selection for early flowering, rapid seed maturation, absence of seed dormancy, small plant size, and high female fertility (*7*). Despite its history of long-term artificial selection, the population has maintained considerable genetic variation and has been used to examine the genetic basis of a wide range of traits and constraints to adaptive evolution [for example, (*8*, *9*)].

Future studies should examine how frequently and how rapidly evolutionary trajectories of natural plant populations are affected by changes in pollination regime and herbivore abundance. This should depend on the strength of selection by pollinators and herbivores and the importance of indirect and nonadditive effects (*10*, *11*). It will also depend on available genetic variation in traits subject to selection. Do natural populations harbor genetic variation that allows them to adapt rapidly to changes in pollination regime and abundance of herbivores? The extent to which adaptive evolution is constrained by genetic correlations among traits affecting these interactions is also unclear. Such investigations should improve predictions of the evolutionary response of plant populations to global environmental change, which involves strong effects on biotic interactions in a wide range of systems. ■

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EVOLUTION

Lost in the fire

Thyroid hormones tip the balance between regeneration and temperature regulation

By **Silvia Marchianò**[1,2,3] *and* **Charles E. Murry**[1,2,3,4,5]

Like all mammals studied, humans are born with considerable regenerative capacity, but for unknown reasons, this is lost in early childhood (*1*). For example, in adulthood, human hearts and brains have minimal ability to regenerate after injury, and age-associated diseases of cell deficiency are major causes of human death. By contrast, amphibians, fish, and invertebrates can regenerate throughout their lives (*2*). This comparative biology has captivated researchers, who hope that understanding natural regeneration might lead to clinical treatments, such as for heart disease (*2*). On page 184 of this issue, Hirose *et al.* (*3*) identify thyroid hormone as a culprit in preventing mammalian heart regeneration. By comparing multiple mammalian species, they propose that regeneration was lost as a trade-off for the ability to regulate body temperature (endothermy).

Natural heart regeneration involves the division of preexisting cardiac muscle cells (cardiomyocytes), rather than the generation of new muscle from stem cells (*4*, *5*). As the mammalian heart matures postnatally (first week in mice and first decade in humans), cardiomyocytes undergo a final round of DNA synthesis with no cell division (*6*). Most adult cardiomyocytes, therefore, have multiple copies of the genome (polyploid), with only a minority remaining diploid (containing a single copy of the genome) (*7*). By contrast, cardiomyocytes of regenerative animals are almost entirely diploid (*8*). Diploid cardiomyocytes divide more frequently, whereas polyploidy inhibits regeneration (*8*, *9*). Thus, logical questions are what causes

[1]*Department of Pathology, University of Washington, 850 Republican Street, Seattle, WA 98109, USA.* [2]*Center for Cardiovascular Biology, University of Washington, 850 Republican Street, Brotman Building, Seattle, WA 98109, USA.* [3]*Institute for Stem Cell and Regenerative Medicine, University of Washington, 850 Republican Street, Seattle, WA 98109, USA.* [4]*Department of Medicine and Cardiology, 1959 NE Pacific Street, University of Washington, Seattle, WA 98195, USA.* [5]*Department of Bioengineering, University of Washington, 3720 15th Avenue NE, Seattle, WA 98105, USA. Email: murry@uw.edu*

polyploidy and can it be manipulated to allow regeneration?

Hirose *et al.* tackled these questions by evaluating the percentage of diploid cardiomyocytes across 41 species. Adult fish, amphibians, and reptiles are mostly (77 to 98%) diploid. Notably, there is a wide range in mammals: Bowhead whales possess 34 to 59% diploid cardiomyocytes, whereas humans have only 4% diploid cardiomyocytes. When correlating the frequency of diploid cardiomyocytes with multiple biometric parameters, they found that ploidy is independent of body weight, heart rate, and blood pressure but strongly correlates with body temperature.

Endothermy, the ability to regulate body temperature within a narrow range, is restricted to birds and mammals (and some species of fish). Amphibians, reptiles, and most fish are ectothermic, meaning that their body temperature is dependent on the environment. As a result, the metabolic rates of ectothermic animals are an order of magnitude less than in endotherms (*10*). In mammals, within minutes of birth, the cold temperature of the environment activates thermogenesis, increasing heat production and metabolic rate to maintain a constant body temperature (*11*). Hirose *et al.* found that as temperature and metabolic rate increase, there is a linear decrease in the numbers of diploid cardiomyocytes. This suggests that there is a mechanistic link between metabolic rate and cardiac regeneration.

Thyroid hormones are important regulators of metabolism and development, and they are thought to drive acquisition of endothermy during development (*12*). Indeed, ectothermic animals exhibit lower concentrations of thyroid hormone compared with endothermic animals. Moreover, soon after birth, the amount of circulating thyroid hormone in neonatal mice increases up to 50-fold (*13*). Hirose *et al.* demonstrated that impairing thyroid hormone signaling by either pharmacological inhibition or gene-editing approaches caused an increase in diploid cardiomyocytes from 9 to 30% in mice at postnatal day 14. Moreover, blocking thyroid signaling increased cardiomyocyte proliferative rates, such that the hearts of these mice were 37% larger than controls and contained around twice as many cardiomyocytes.

The increased regenerative capacity of the gene-edited mice was demonstrated by inducing a myocardial injury in the adult mice. At 28 days postinjury, these mice exhibited a 10-fold increase in proliferating mononucleated and diploid cardiomyocytes, associated with a 62% reduction of scar area and 11% improvement in cardiac function. Hirose *et al.* also demonstrated that thyroid signaling could inhibit natural heart regeneration in zebrafish. Administering thyroid hormone to zebrafish with injured hearts increased cardiomyocyte polyploidy by fivefold, accompanied by a 45% reduction in proliferation, impaired regeneration, and healing by scar formation.

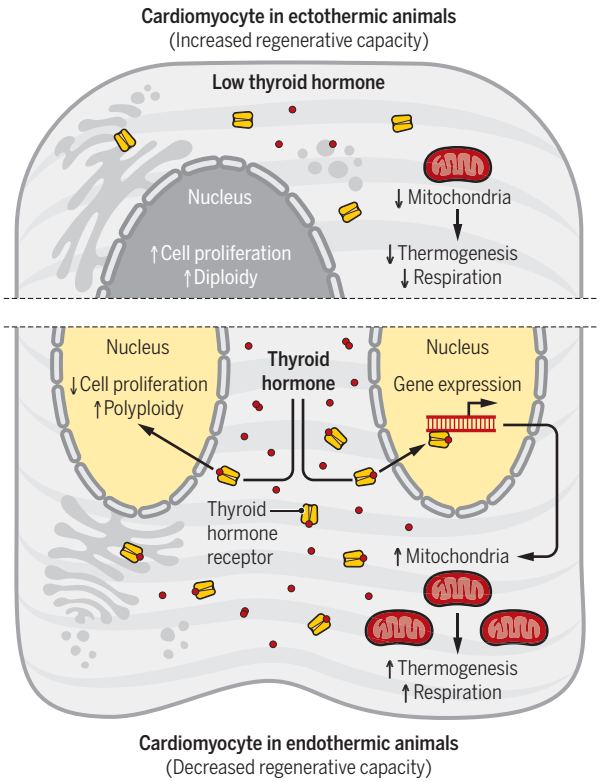
The study of Hirose *et al.* establishes the role of thyroid hormone as an important regulator of cardiomyocyte ploidy and regenerative capacity across the animal kingdom (see the figure). These results agree with previous studies in human pluripotent stem cell–derived cardiomyocytes, in which treatment with thyroid hormone reduced cardiomyocytes' cell-cycle activity (*14*). However, manipulating the amount of thyroid hormone as a clinical approach to improve cardiac regeneration is challenging. Indeed, low concentrations of circulating thyroid hormones (hypothyroidism), due to thyroid gland dysfunction, alter cardiac gene expression and increase vascular resistance, both resulting in reduced cardiac performance. Moreover, failing hearts may be functionally hypothyroid, owing to impaired local signaling. Thus, thyroid hormone supplementation has been proposed as a treatment for heart failure patients (*15*). This emphasizes the need for a more detailed understanding of how thyroid signaling regulates regenerative capacity. It will be interesting to evaluate the regenerative capacity of mature cardiomyocytes in which thyroid hormone signaling is impaired in adulthood.

Frogs, whose metamorphosis requires a transient increase in thyroid hormones, are still capable of heart and skin regeneration, but not of limb or spinal cord. Humans, by contrast, can regenerate part of the liver and the skin but not other organs (including the heart). Hirose *et al.* demonstrate that, although thyroid hormone inhibits regeneration in zebrafish hearts, it does not limit their fin regeneration, demonstrating tissue specificity. Further experiments to understand differences among different organs and different species may give new insight into regenerative capacity.

Why is regeneration lost in adult mammalian hearts? Hirose *et al.* suggest that the loss of regenerative capacity represents a trade-off for acquisition of endothermy. But it is not clear why endothermy should be incompatible with regeneration. Endothermy allows for faster and more constant biochemical flux rates, and this has allowed humanity to spread over a wide geographical distribution. Does the capacity to regenerate somehow reduce cardiac fitness? Identifying pathways, other than thyroid hormones, that are "extinguished" by evolution could lead to the discovery of new targets to promote tissue regeneration. ■

Regenerative capacity in the animal kingdom

Endotherms gained thyroid signals over the course of evolution and became thermogenic, perhaps in a trade-off with regeneration capacity.



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Silvia Marchianò and Charles E. Murry

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