ern fish grow more slowly because summers are longer and winters milder.

The phenotype-centric approach assumes that when phenotypic traits are selected, corresponding changes at the molecular level follow suit (*5*). This mechanism is easiest to grasp for continuous traits—phenotypes that display a range of presentations, such as body height or growth rate—as these are often influenced by the joint effects of perhaps hundreds of genes. If there is, for example, a selective advantage of fast growth, then individuals having more of the gene variants (alleles) for fast growth also produce more offspring, and alleles for fast growth will accumulate.

The breathtaking diversity of life suggests that genomic constraints on the path of evolution are less restrictive if genes can be recombined and reshuffled over many generations. But with the current rate of anthropogenic change, evolution happens over just a few generations. When evolution approaches maximum rates, or when only a few genes in specific pathways are involved (*6*), it begins to matter where the genes are physically positioned on chromosomes and whether the genes affect multiple nodes in the complex biochemical network of a cell. Thus, scientists require a second, DNA-centric approach that views evolution from the bottom up (from genes to organisms). This perspective adds a focus on the effects of distinct alleles and their interactions, which can make evolutionary trajectories deviate from those predicted by phenotype-centric methods. Under strong selection, certain alleles might confer substantial benefits to their bearers and, over a few generations, spread through the population like a brushfire (a so-called selective sweep). Another gene that happens to reside in a nearby chromosomal location can hitchhike to fame, and its effects on the organism can influence the evolutionary outcome.

The genomic changes in the 2002 silverside study are of interest because selection was strong and evolution rapid. Large fish were removed from two populations (Down1 and Down2), and after four generations, the fish were markedly smaller. The opposite happened when small fish were harvested; and when harvesting was random, no change in body size occurred.

Therkildsen *et al.* pulled the fish from 2002 out of the freezer and searched their genomes for changes in genetic markers that accompanied the evolved size differences. In all of the experimental populations, thousands of genetic markers spread over nearly every chromosome changed in frequency. These results support the phenotype-centric view of polygenic evolution and are not surprising for a trait such as growth, which depends on multiple physiological processes. There was also strong evidence that attributes of the physical DNA molecule altered evolutionary outcomes. In the Down2 experimental tank, one individual had a chromosome variant typical of slow-growing fish from the southern range of this species' distribution in the wild. This variant swept through the Down2 experimental population to near-total dominance, and all of the alleles on this large chromosomal segment were inherited by the fourth generation. This observation is well explained by the DNA-centric view of evolution but would come as a surprise to a researcher solely relying on phenotype-centric methods.

Therkildsen *et al.* also demonstrated how maintaining genetic diversity might be crucial to conserving species diversity. Rapid evolution of growth was made possible in part by preexisting growth differences throughout the species' natural range. Alleles that became common in fast-growing laboratory populations were shared by northern, fast-growing fish in the wild, and populations that evolved a slow-growth phenotype accumulated southern-type alleles. Without the broad array of alternative alleles in the starting populations, the rate of evolution would have been orders of magnitude slower because new genetic material would have had to arise from random mutations.

It is worrisome how quickly alleles disappeared during the short experiment. Alleles that strongly increased in frequency in one population did not necessarily change in parallel populations subjected to the same treatment. Thus, several genomic configurations yielded similar phenotypes (*7*), and different alleles were lost in different populations. Such chance events are typical of small populations. However, genetic variance eroded even faster in the selected silverside lines because it was similar individuals that survived and bred. This suggests that conservationists who assess a population as threatened because of its small size should extend this concern to somewhat larger populations that were recently or are currently under strong selection. ■

REFERENCES AND NOTES

1. D. O. Conover, S. B. Munch, *Science* **297**, 94 (2002).
2. N. O. Therkildsen *et al.*, *Science* **365**, 487 (2019).
3. J. S. Santangelo, L. R. Rivkin, M. T. J. Johnson, *Proc. Biol. Sci.* **285**, 20181529 (2018).
4. S. A. Arnott, S. Chiba, D. O. Conover, *Evolution* **60**, 1269 (2006).
5. A. Grafen, in *Behavioural Ecology: An Evolutionary Approach*, J. R. Krebs, N. B. Davies, Eds. (Blackwell Scientific, ed. 2, 1984).
6. N. M. Reid *et al.*, *Science* **354**, 1305 (2016).
7. J. Giske *et al.*, *Proc. Biol. Sci.* **281**, 20141096 (2014).
8. M. Heino M, B. Diaz Pauli, U. Dieckmann, *Ann. Rev. Ecol. Evol. Syst.* **46**, 461 (2015).

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MICROBIOLOGY

Walk on the wildling side

Wild microbiota in inbred mice is a new tool for preclinical studies of human disease

By **Samuel Philip Nobs**[1] *and* **Eran Elinav**[1,2]

Animal models are an important tool in investigating molecular mechanisms of disease pathogenesis and in developing therapeutic approaches for human disease. In many instances though, success in preclinical experiments cannot be translated into the human setting (*1*). One potential explanation for this discrepancy between animal model and human experimental outcomes may involve the microbiota, a large ecosystem of bacteria, fungi, protozoa, and viruses that colonize mucosal surfaces in the human body, particularly the gastrointestinal tract (*2*). Differences in microbiota diversity, resilience, and presence of pathogens between laboratory animals and other "wild" mammals may lead to a limited reproducibility of animal experimentation when attempted in different localities or when used as models of human disease (*3*). On page 461 of this issue, Rosshart *et al.* (*4*) generate a more physiologically relevant preclinical mouse model by combining the diversity of the microbiota found in wild mice with the genetic uniformity of laboratory animals.

It has been suggested that the microbiota is of major importance for human health, regulating the development of many diseases, such as obesity, asthma, inflammatory bowel disease, and cancer (*5*). Interindividual differences in the microbiota may explain some phenotypic variability in humans, such as responses to food (*6*), cancer immunotherapy (*7*, *8*), or drugs (*9*). The microbiota has been shown to influence multiple organ systems, including the immune system (*5*), and its manipulation or the manipulation of its metabolic products is considered a promising avenue for therapy of human disease (*10*).

Rosshart *et al.* created "wildling" mice by inverse germ-free rederivation, trans-

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planting embryos from C57BL/6 mice, the most commonly used laboratory mouse strain, into wild surrogate dams (mothers) (see the figure). The resulting offspring can be kept in a normal animal facility, but they harbor a microbiota that more closely resembles that of wild mice at multiple body sites, including the gut, skin, and vaginal tract. Analyses also revealed that the microbiota resemblance to wild mice is not only true for bacteria but also for other microbes, such as fungi and even viruses.

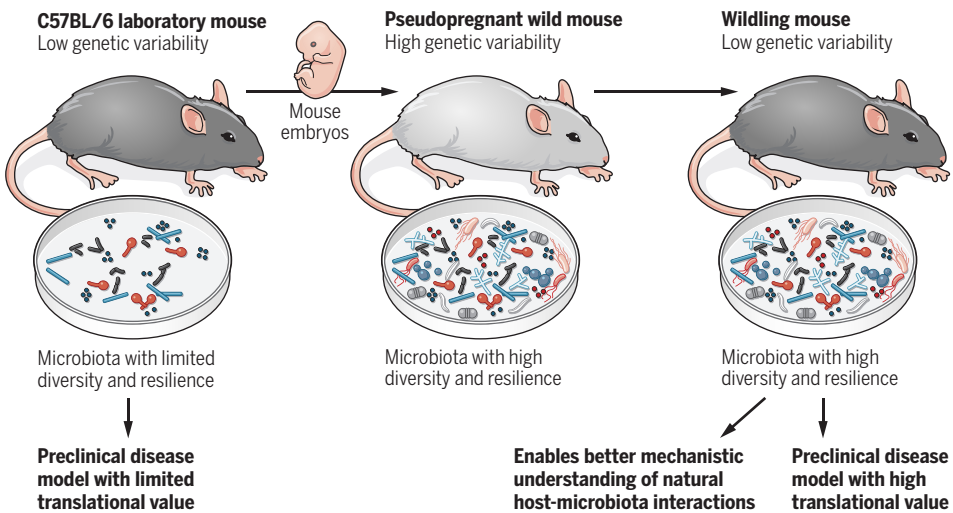
Rosshart *et al.* demonstrate that the presence of a microbiota more similar to that of wild mice has a major impact on the makeup of the tissue-specific immune system in wildling mice in terms of cell composition and expression of immune-related genes. Furthermore, the wildling microbiota is markedly stable over time and persists through multiple generations of breeding. In contrast to the microbiota of laboratory mice, the wildling microbiota is resilient to external challenges such as treatment with broad-spectrum antibiotics or a change in diet, recovering quickly to its original wildling configuration after the perturbation is stopped. This more physiologically relevant microbiota appears to be uniquely suited to the mouse gastrointestinal environment because it is able to replace the microbiota of laboratory mice from different commercial vendors upon cohousing. The authors also demonstrated the potential of the wildling model as an improved preclinical tool, at least for immunotherapy. They recapitulated the failures of two immunotherapy clinical trials in humans using wildling mice, demonstrating that the immune phenotype and survival of wildling mice much more closely resemble those of humans than those of laboratory mice. This implies that a more complex microbial ecology is key to predicting immune-mediated outcomes of therapy.

This study has a number of key implications for better adaptation of mouse preclinical experimentation for the study of human diseases, as well as for investigating the role of the microbiota in a disease context. It paves the way for a more human-relevant preclinical approach by providing an improved in vivo tool harboring an immune system that more closely reflects human biology, while preserving the utility of inbred mice. The study reveals that the degree of microbiota-mediated immune-phenotype regulation in wildling mice is strongly tissue-specific, suggesting that the intrinsic genetic makeup of a tissue is complemented by environmental modulation in shaping an organotypic immune response. This tissue-specific immune configuration is somehow modulated by the diverse wildling microbiota that serves as a relay between unidentified extrinsic environmental factors and the intrinsic sterile host milieu. The bacterial wildling gut microbiota maintains an interindividual diversity, similar to that of the human gut microbiota, which could account for tissue-specific and individual-specific variations. The mechanisms by which the wildling microbiota contributes to phenotypic variability may be important to investigate, as these may explain both variations in healthy traits, as well as susceptibility to development of different manifestations of complex diseases despite genetic similarities between human patients. Even interindividual variability of the microbiota appears to be tissue-specific, because the vaginal tract microbiota remains very similar between wildling and wild mice, whereas the gut microbiota of wildling animals features significant differences in composition between animals. Differences in microbiota composition between wild and wildling mice may stem from genetic differences affecting the microbial configuration, or from lifestyle changes noted between cage-dwelling wildling mice and wild mice (*11*).

This new animal model may better serve research of host-microbiota responses to differing environmental exposures (*11*), such as diets, xenobiotics, and microbiota-associated diseases. Furthermore, wildling mice provide an opportunity to study the effects of microorganisms other than bacteria, such as fungi and viruses. The incorporation of these microorganisms into a more natural microbiota configuration is likely to affect both metabolic and immune phenotypes in wildling mice, thereby influencing disease development and responses to therapy. It will be exciting to use this "wildized" microbiota setting to study the extent and mechanism of contribution of the fungome and virome to host homeostasis and disease susceptibility. Wildling mice will likely provide an elegant and more robust preclinical model to predict success in human clinical trials, by exhibiting more-similar responses to therapeutics than traditional laboratory animals. Incorporation of this approach into preclinical research could reduce the number of failed clinical trials, prevent unnecessary risks for patients, reduce unnecessary animal use, and preserve research resources. ■

Wildling mice: A new preclinical tool to study human disease

Wildling mice combine the low genetic diversity of inbred laboratory mice with a wild mouse microbiota harboring bacteria, fungi, and viruses. This generates a more translationally relevant animal model of human disease for studying host-microbiota interactions, as well as for predicting the outcomes of human clinical trials.



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REFERENCES AND NOTES

1. M. McNutt, *Science* **343**, 229 (2014).
2. R. Knight *et al.*, *Annu. Rev. Genomics Hum. Genet.* **18**, 65 (2017).
3. D. Masopust, C. P. Sivula, S. C. Jameson, *J. Immunol.* **199**, 383 (2017).
4. S. P. Rosshart *et al.*, *Science* **365**, eaaw4361 (2019).
5. S. P. Nobs, T. Tuganbaev, E. Elinav, *EMBO Rep.* **20**, e47129 (2019).
6. D. Zeevi *et al.*, *Cell* **163**, 1079 (2015).
7. V. Matson *et al.*, *Science* **359**, 104 (2018).
8. V. Gopalakrishnan *et al.*, *Science* **359**, 97 (2018).
9. M. Zimmermann, M. Zimmermann-Kogadeeva, R. Wegmann, A. L. Goodman, *Nature* **570**, 462 (2019).
10. J. Suez, N. Zmora, E. Segal, E. Elinav, *Nat. Med.* **25**, 716 (2019).
11. D. Rothschild *et al.*, *Nature* **555**, 210 (2018).

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