close to the substrate, is "softened" by steric effects due to the structure of the liquid crystalline molecules. From the morphologies visible on a micrometer scale, together with knowledge about film thicknesses, they extracted the form of the interaction profile on a nanometer scale. They thus were able to infer the intermolecular interactions acting in their films just by looking through a microscope. These experimental findings are also in accordance with three-dimensional numerical simulations (*10*). Unfortunately, it is still not clear what kind of alternative process is able to produce in polystyrene films almost the same patterns found in gold films. Only a thorough analysis of the hole patterns based on Minkowski measures reveals differences, but it does not explain where they come from.

Why is an experimental proof of spinodal dewetting in thin films of so much importance? Because its existence implies that long-range forces over the distance of many molecular diameters can create patterns on macroscopic length scales. Furthermore, this process is an intrinsic phenomenon that can hardly be avoided without changing the system. Recent experiments show that these forces can be sufficiently strong to even destabilize thin liquid films confined between two solid walls, where one wall consists of a thin solid film. Thus, for many applications involving thin films or, in a more general sense, two parallel interfaces separated by some medium, the understanding and the control of the relevant molecular interactions are highly desirable.

What are the prospects for such control? The influence of ubiquitous long-range dispersive forces at separation distances up to 100 nm and more can be drastic, although these forces are, at such distances, already rather weak. This is largely neglected because directly at a surface or an interface they are mostly weak compared with specific interaction of short range. For example, long-range van der Waals forces (per unit area) at a distance of 100 nm amount to less than 1 Pa. Nonetheless, they can rupture 100-nm films, even if these films are "glued" to the substrate by specific short-range interactions. Experiments by Herminghaus *et al.* and others (*1*, *7*) show that it is not possible to avoid such instabilities by a simple surface treatment or surface modification of the substrate. Improving adhesion between substrate and film by grafting polymers onto the substrate or by adding a thin layer of adhesion promoter [such as the chromium layer used by Herminghaus *et al.* (*1*)] will only stabilize films that are sufficiently thin, namely, about the thickness of this additional layer. Thus, we may be forced to reconsider the influence of long-range forces, especially at large separations, in areas ranging from physical properties of thin films (where processes like phase transitions or phase separation differ from the behavior in the bulk) to biology (cell adhesion or deformation).

So, if one cannot avoid this problem, can it be of potential use? As indicated by Herminghaus *et al.* (*1*), we can learn about the intermolecular forces in films confined by a liquid-fluid interface. At present, especially for strong attractive forces, there are no other techniques available that have such a possibility. One may also consider using this creative power of intermolecular forces to produce regular patterns, probably by employing some lateral confinement in competition with the thickness-dependent characteristic wavelength of the instability. Structuring of multilayer systems by some intermediate unstable liquid layer seems also possible. Consequently, I expect that many groups will intensify their efforts in these directions and not just because of the aesthetic aspects of the evolving patterns.

References

1. S. Herminghaus *et al.*, *Science* **282**, 916 (1998).
2. A. Scheludko, *Adv. Colloid Interface Sci.* **1**, 391 (1967).
3. A. Vrij, *Discuss. Faraday Soc.* **42**, 23 (1966).
4. G. Reiter, *Phys. Rev. Lett.* **68**, 75 (1992).
5. K. Jacobs, S. Herminghaus, K. R. Mecke, *Langmuir* **14**, 965 (1998).
6. R. Xie, A. Karim, J. F. Douglas, C. C. Han, R. A. Weiss, *Phys. Rev. Lett.* **81**, 1251 (1998).
7. G. Reiter *et al.*, in preparation.
8. F. Brochard-Wyart and J. Daillant, *Can. J. Phys.* **68**, 1084 (1990).
9. J. Bischof, D. Scherer, S. Herminghaus, P. Leiderer, *Phys. Rev. Lett.* **77**, 1536 (1996).
10. A. Sharma and R. Khanna, *Phys. Rev. Lett.* **81**, 3463 (1998).

PERSPECTIVES: EVOLUTIONARY GENETICS

The Causes of Haldane's Rule

Michael Turelli

Speciation, the splitting of single evolutionary lineages into reproductively isolated groups, remains one of the most elusive phenomena in evolution. The process requires thousands to millions of years, and we are confronted with a vast array of demonstrable contributors—geographical isolation, natural selection, sexual selection, and changes in karyotype, to name several—whose relative importance is difficult to untangle (*1*). The last 10 years have brought noticeable progress in the genetics of speciation—progress that has come because evolutionary geneticists have focused largely on one aspect of speciation: the production of inviable and sterile hybrids and Haldane's rule.

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The most tantalizing regularity in animal speciation, this rule derives from Haldane's (*2*) observation that "When in the F_1 [first generation] offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic; XY, XO, or ZW] sex." Haldane's rule is a way station through which almost all pairs of animal species pass on their way to producing completely inviable or sterile hybrids (*1*). In a study on page 952 of this issue, Presgraves and Orr (*3*) provide near-definitive data that address the causes of Haldane's rule. Contrary to the classic Popperian formula in which science marches forward over the corpses of rejected hypotheses, their report provides empirical support for both of the most widely accepted explanations.

Haldane's rule holds for 99% of 223 cases of sex-specific hybrid sterility and 90% of 115 cases of sex-specific hybrid inviability (*3*, *4*) (see the figure). If stretched to include cases in which hybrid females and males differ only quantitatively in fertility or viability, the rule extends to even more animals (*4*) and some dioecious plants (*5*). Its generality suggests that a common evolutionary force may underlie speciation in many different groups. The fact that Haldane's rule predicts inviable or sterile (heterogametic) males in Diptera and mammals, but inviable or sterile (heterogametic) females in Lepidoptera and birds, implies a critical role for the sex chromosomes in this intermediate step in speciation, rather than just for gender per se.

For more than 40 years, most evolutionists accepted an X chromosome–based explanation of Haldane's rule proposed by Muller (*6*). Muller's hypothesis built on Dobzhansky's (*7*) insight that genetic changes beneficial or harmless in one genetic background may be deleterious in another genetic background, because of negative interactions that had not been screened by natural selection. Thus, over time—on the order of 10^5 to 10^6 years—isolated populations accumulate increasing numbers of genetic changes that may be advantageous or neutral within species but that produce

sterility or inviability in hybrids. Muller recognized that deleterious interactions between X-linked loci and autosomal loci would affect the sexes differently. In particular, he argued that X-linked incompatibilities, if partially recessive, would more seriously afflict the sex with only one X, as hybrids of that sex would fully display the deleterious recessive effects. This explanation for Haldane's rule—recently elaborated and dubbed the "dominance theory" (*8*, *9*)—held sway until an influential 1985 experiment (*10*) reawakened interest in Haldane's rule.

Coyne (*10*) studied two *Drosophila* hybridizations that obey Haldane's rule for sterility. He created "unbalanced" hybrid females—females who inherited both of their X's from their mother (by using attached-X stocks in which females' X chromosomes are connected and therefore transmitted together to their daughters). He argued that the sterility of hybrid males should imply sterility of the unbalanced hybrid females under Muller's theory, because these females' X-autosome incompatibilities are just as severe as those of hybrid males. Yet unbalanced females were fertile in both tests. This led to a temporary eclipse of the dominance theory and a scramble for alternatives (*11*).

Several hypotheses were proposed (*4*, *12*), but only one has found much support: the "faster male" theory of Wu and Davis (*13*). This theory, which attempts to explain only Haldane's rule for sterility in male heterogametic species, is based on a fundamental distinction between the genetics of sterility and the genetics of inviability. Coyne's test implicitly assumed that incompatibilities causing hybrid male and female sterility are identical or at least accumulate at similar rates. Yet we know from work in *Drosophila* that, although most lethal mutations within species kill both sexes, most sterile mutations are sex-specific. Thus, we might expect different sets of loci to produce male and female hybrid sterility. These loci might well be subject to sex-specific evolutionary pressures and thus may evolve at different rates. Males in particular are often subject to intense sexual selection that contributes to rapid divergence of male phenotypes ranging from plumage to genitalia, possibly accelerating the rate of evolution at loci that produce male-specific sterility. These observations motivate the faster-male hypothesis—that Haldane's rule for sterility in male-heterogametic species follows from the faster accumulation of male-sterilizing than of female-sterilizing incompatibilities. This mechanism is not a universal explanation for Haldane's rule, as it explains neither Haldane's rule for inviability (assuming that the same incompatibilities afflict both sexes) nor Haldane's rule for sterility in female-heterogametic species (in such species, the males still show accelerated evolution for sexual traits, but hybrid females are differentially sterile or inviable). Nevertheless, the faster-male hypothesis gained strong experimental support from two recent genetic introgression experiments on pairs of *Drosophila* that obey Haldane's rule for sterility (*14*). Both experiments found that hybrid genotypes created by inserting small segments of the *D. mauritiana* genome into *D. simulans* were far more likely to be male-sterile than female-sterile (or inviable in either sex).

EXAMPLES OF HALDANE'S RULE

	Hybrid females	Hybrid males
Drosophila	XX	XY sterile, inviable
Anopheles	XX	XY sterile, inviable
Aedes	X_fX_f	X_fX_m* sterile
Mammals	XX	XY sterile
Birds	ZW sterile, inviable	ZZ
Lepidoptera	ZW sterile, inviable	ZZ

*X_f and X_m denote sex chromosomes that are largely homologous, with only a small sex-determining region.

Although persuasive, the introgression results involved only one small clade. These experiments are too labor-intensive to replicate broadly, and they do not critically evaluate the relative contributions to Haldane's rule of faster-male evolution and dominance. To test the generality of both theories, Presgraves and Orr (*3*) analyzed published data from 174 mosquito hybridizations, 34 from the genus *Aedes* and 140 from the genus *Anopheles*. In *Aedes*, a small region of the sex chromosomes determines gender, with the rest of the X and Y chromosomes being homologous and fully functional; thus, almost all genes on the sex-determining chromosomes show autosome-like patterns of inheritance. The second group of mosquitoes, *Anopheles*, has typical X-Y sex determination, with a genetically inert Y.

Presgraves and Orr's (*3*) central insight is that faster-male evolution can produce Haldane's rule for sterility in male-heterogametic species, whether or not the males have "typical" hemizygous sex chromosomes. As predicted by the faster-male theory, *Aedes* hybridizations clearly follow Haldane's rule for sterility, with sterile males appearing in all 11 cases of sex-specific hybrid sterility. Without a large region of the X chromosome being effectively hemizygous, Muller's dominance mechanism should be ineffective in *Aedes*. As predicted by the dominance theory, *Aedes* does not display Haldane's rule for inviability. In contrast, in *Anopheles*, where both the dominance and faster-male mechanisms should act, 21 of the 24 examples of sex-limited hybrid inviability follow Haldane's rule. *Anopheles* also obey Haldane's rule for sterility in all 56 cases of sex-specific hybrid sterility. Moreover, a larger overall fraction of *Anopheles* than of *Aedes* hybridizations follow Haldane's rule, as expected given that in *Anopheles* two Haldane's rule–producing forces are acting together. Thus, Presgraves and Orr have found strong evidence supporting both of the leading explanations for Haldane's rule.

Our laboratory has provided complementary support for the dominance theory in a recent study of published data from 125 *Drosophila* hybridizations (*15*). Of the 125 species pairs, 81 have "small" X chromosomes (including about 20% of the genome) and 44 have "large" X chromosomes (about 40% of the genome). We compared the association between hybrid viability/fertility and "genetic distances," which crudely estimate evolutionary divergence times, in large-X and small-X pairs. If hybrid dysfunction involves many incompatibilities among loci scattered throughout the genome, large-X pairs should experience more X-autosome problems than small-X pairs for a given amount of genetic divergence. Thus, the dominance theory predicts that hybrid males from large-X pairs will be less fit than hybrid males from small-X pairs, all else being equal. As predicted, Haldane's rule occurs at smaller average genetic distances between large-X pairs than between small-X pairs.

These new results should bolster the conclusion that both faster-male evolution and dominance contribute to Haldane's rule. Of these explanations, however, only the dominance theory can explain Haldane's rule for

SOURCE: (3, 4)

inviability in any group or Haldane's rule for sterility in female-heterogametic taxa. The dominance theory also provides a natural explanation for two additional phenomena: The repeated finding that the X chromosome appears to contribute disproportionately to hybrid inviability and sterility (the "large X effect") (*11*) and the absence of Haldane's rule for sterility in mammals [see (*9*) for an explanation]. Conversely, faster-male evolution unquestionably plays a role in Haldane's rule for sterility in *Drosophila* and mosquitoes.

What remains to be done? We still know too little about the genes that cause postzygotic isolation. We lack, for instance, direct genetic analyses assessing whether X-linked incompatibilities in hybrids behave recessively, although several lines of evidence suggest that they do (*4*, *9*, *12*, *15*). The demonstrated power of analyzing Haldane's rule in diverse taxa should encourage researchers to look more carefully beyond Diptera. New insights may emerge from dioecious plants, such as *Silene*, with heteromorphic sex chromosomes (*5*). We also need more genetic analyses of Haldane's rule in female-heterogametic species. If the faster-male mechanism is working in these taxa, as suggested by the pervasive phenotypic evidence for sexual selection in birds and butterflies, it should oppose the appearance of Haldane's rule for sterility. In female-heterogametic taxa, therefore, we might expect to see a relative excess of hybrids producing Haldane's rule for inviability rather than sterility. In fact, the most recent data (*3*, *4*) show that 69% of the 84 examples of Haldane's rule in birds and Lepidoptera involve inviability, in contrast to only 19% of 217 examples in Diptera. Birds and Lepidoptera pose an additional challenge because their sex chromosomes are generally much smaller than those of Diptera. In these groups, how can dominance give rise to Haldane's rule when there are so few X-linked loci (*9*)? Although the main causes of Haldane's rule now seem clear, many ancillary questions await further analyses.

References and Notes

1. J. A. Coyne and H. A. Orr, *Philos. Trans. R. Soc. London Ser. B* **353**, 287 (1998).
2. J. B. S. Haldane, *J. Genet.* **12**, 101 (1922).
3. D. C. Presgraves and H. A. Orr, *Science* **282**, 952 (1998).
4. C. C. Laurie, *Genetics* **147**, 937 (1997).
5. D. R. Taylor, *Heredity* **74**, 518 (1993).
6. H. J. Muller, in *The New Systematics*, J. Huxley, Ed. (Clarendon, Oxford, 1940), pp. 185–268; *Biol. Symp.* **6**, 71 (1942).
7. Th. Dobzhansky, *Genetics and the Origin of Species.* (Columbia Univ. Press, New York, 1937), p. 256.
8. H. A. Orr, *Evolution* **47**, 1606 (1993).
9. M. Turelli and H. A. Orr, *Genetics* **140**, 389 (1995).
10. J. A. Coyne, *Nature* **314**, 736 (1985).
11. ——— and H. A. Orr, in *Speciation and Its Consequences*, D. Otte and J. A. Endler, Eds. (Sinauer, Sunderland, MA, 1989), pp. 180–207.
12. H. A. Orr, *Annu. Rev. Ecol. Syst.* **28**, 195 (1997); C.-I. Wu, N. A. Johnson, M. F. Palopoli, *Trends Ecol. Evol.* **11**, 281 (1996).
13. C.-I. Wu and A. W. Davis, *Am. Nat.* **142**, 187 (1993).
14. J. R. True, B. S. Weir, C. C. Laurie, *Genetics* **142**, 819 (1996); H. Hollocher and C.-I. Wu, *ibid.* **143**, 1243 (1996).
15. M. Turelli and D. J. Begun, *ibid.* **147**, 1799 (1997).

16. I thank J. A. Coyne, C. H. Langley, H. A. Orr, T. W. Schoener, and M. M. Turelli for discussions and comments.

PERSPECTIVES: DEVELOPMENT

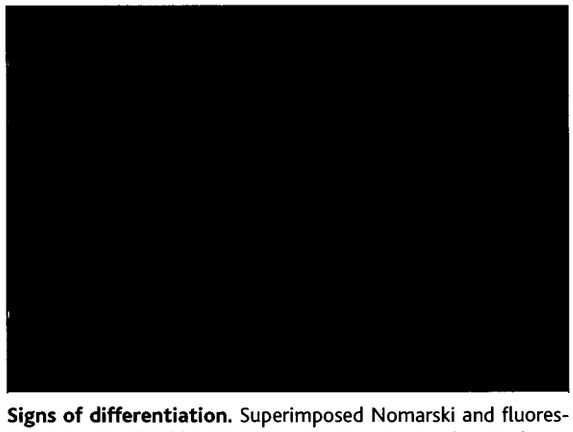
How Cyanobacteria Count to 10

Robert Haselkorn

The cyanobacterium *Anabaena*, although a prokaryote, develops elegantly in a way that more closely resembles eukaryotic development. About every tenth cell in its linearly arranged string of photosynthetic vegetative cells differentiates into an anaerobic, nitrogen-fixing heterocyst, as shown in the figure. This differentiation is provoked by an environmental cue—the absence of a fixed nitrogen source in the growth medium. Genetic analysis of mutants that cannot differentiate properly has led to a complex picture of interacting gene products that control the frequency of differentiating cells along each filament. In this issue on page 935, Yoon and Golden show that the gene *patS*, which encodes a 17–amino acid peptide, contributes to the spacing pattern by preventing the differentiation of cells between two heterocysts (*1*). Heterocyst differentiation can be suppressed completely by the external addition of the carboxyl-terminal five amino acids of the peptide Arg-Gly-Ser-Gly-Arg.

Control of differentiation by a small peptide sounds distinctly eukaryotic. Many other aspects of *Anabaena* heterocyst differentiation also recall or, in some cases, anticipate, signaling processes in eukaryotes. For example, some of *Anabaena*'s genes encoding nitrogenase proteins contain intervening DNA elements that have to be excised during each heterocyst differentiation, in a process reminiscent of the rearrangements of immunoglobulin genes in developing B lymphocytes (*2*). *Anabaena* also houses several serine-threonine–type protein kinases (*3*), a very large number of histidine kinases (*4*), probably a tyrosine kinase, and certainly a phosphotyrosine phosphatase (*5*).

How does a suppressing peptide fit into the current scheme for pattern regulation in *Anabaena*? We already know the identity of a few gene products that are required for heterocysts to differentiate. The protein acting earliest in the process, NtcA, is a DNA-binding factor required for transcription of numerous genes encoding components for nitrogen transport and utilization. NtcA is related by sequence to the cyclic AMP–binding protein of *Escherichia coli* (*6*). Among the genes regulated by NtcA is one called *hetR*, whose product is required for heterocyst differentiation (*7*). The figure shows the distribution of *hetR* transcripts, revealed by a fusion of the *hetR* promoter to the *gfp* gene, which encodes the green fluorescent protein. The filaments were photographed 1 day after nitrogen concentrations were decreased, when mature heterocysts display their characteristic spacing, about one every ten cells. The striking result is that the *hetR* gene is transcribed only in the differentiating cells. Exactly the same result is reported for another gene, *patS*, in the new work by Yoon and Golden (*1*). However, there is a critical difference, established both by the mutant phenotypes and the phenotypes of cells carrying extra copies of either the *hetR* or *patS* genes. That is, cells with mutant HetR do not initiate differentiation, whereas cells with mutant PatS dif-



Signs of differentiation. Superimposed Nomarski and fluorescent images of wild-type *Anabaena* PCC 7120, taken 25 hours after differentiation was initiated by nitrogen withdrawal. Differentiated heterocysts are marked by expression of green fluorescent protein (GFP) from a plasmid containing the *hetR* gene promoter fused to the GFP coding sequences.

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