

cessful over the long term. However, our limited across-season data suggests that short-sleeping males may actually perform better than do long-sleeping males over the long term, suggesting ongoing sexual selection instead. Ultimately, a greater understanding of potential short- and long-term costs of reproductive sleep loss in pectoral sandpipers may provide insight into the evolution of this extreme behavior, as well as the ongoing debate over the functions of sleep (25) and its relationship to health and longevity in humans (26, 27).

References and Notes

1. C. Cirelli, G. Tononi, *PLoS Biol.* **6**, e216 (2008).
2. D. Dawson, K. Reid, *Nature* **388**, 235 (1997).
3. J. S. Durmer, D. F. Dinges, *Semin. Neurol.* **25**, 117 (2005).
4. A. Rolls *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13305 (2011).
5. V. V. Vyazovskiy *et al.*, *Nature* **472**, 443 (2011).
6. B. A. Klein, A. Klein, M. K. Wray, U. G. Mueller, T. D. Seeley, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 22705 (2010).
7. J. M. Siegel, *Nat. Rev. Neurosci.* **10**, 747 (2009).
8. M. Andersson, *Sexual Selection* (Princeton Univ. Press, Princeton, NJ, 1994).
9. J. Byers, E. Hebets, P. Jeffrey, *Anim. Behav.* **79**, 771 (2010).
10. J. Barske, B. A. Schlinger, M. Wikelski, L. Fusani, *Proc. Biol. Sci.* **278**, 3523 (2011).
11. Materials and methods are available as supplementary materials on Science Online.
12. F. A. Pitelka, R. T. Holmes, S. F. MacLean, *Am. Zool.* **14**, 185 (1974).
13. A. L. Vyssotski *et al.*, *Curr. Biol.* **19**, 1159 (2009).
14. N. C. Rattenborg *et al.*, *PLoS Biol.* **2**, E212 (2004).
15. J. A. Lesku *et al.*, *PLoS ONE* **6**, e23203 (2011).
16. J. A. Lesku, A. L. Vyssotski, D. Martinez-Gonzalez, C. Wilzeck, N. C. Rattenborg, *Proc. Biol. Sci.* **278**, 2419 (2011).
17. S. Leemburg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15939 (2010).
18. T. Fuchs, D. Maury, F. R. Moore, V. P. Bingman, *Biol. Lett.* **5**, 77 (2009).
19. S. G. Jones, E. M. Paletz, W. H. Obermeyer, C. T. Hannan, R. M. Benca, *BMC Neurosci.* **11**, 87 (2010).
20. O. Lyamin, J. Pryslova, V. Lance, J. Siegel, *Nature* **435**, 1177 (2005).
21. O. I. Lyamin, P. R. Manger, S. H. Ridgway, L. M. Mukhametov, J. M. Siegel, *Neurosci. Biobehav. Rev.* **32**, 1451 (2008).
22. C. M. Jung *et al.*, *J. Physiol.* **589**, 235 (2011).
23. S. L. Lima, N. C. Rattenborg, J. A. Lesku, C. J. Amlaner, *Anim. Behav.* **70**, 723 (2005).
24. V. Bachmann *et al.*, *Cereb. Cortex* **22**, 962 (2012).
25. S. Daan, *Sleep Biol. Rhythms* **9**, 1 (2011).
26. D. F. Kripke, R. D. Langer, J. A. Elliott, M. R. Klauber, K. M. Rex, *Sleep Med.* **12**, 28 (2011).
27. J. Horne, *Biol. Psychol.* **87**, 1 (2011).

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Supplementary Materials

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Mutations in the *neverland* Gene Turned *Drosophila pachea* into an Obligate Specialist Species

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Most living species exploit a limited range of resources. However, little is known about how tight associations build up during evolution between such specialist species and the hosts they use. We examined the dependence of *Drosophila pachea* on its single host, the senita cactus. Several amino acid changes in the Neverland oxygenase rendered *D. pachea* unable to transform cholesterol into 7-dehydrocholesterol (the first reaction in the steroid hormone biosynthetic pathway in insects) and thus made *D. pachea* dependent on the uncommon sterols of its host plant. The *neverland* mutations increase survival on the cactus's unusual sterols and are in a genomic region that faced recent positive selection. This study illustrates how relatively few genetic changes in a single gene may restrict the ecological niche of a species.

Losses of enzymatic activities are frequent during evolution (1). For example, humans lost the ability to produce nine amino acids and six vitamins, for which we rely on our diet (2). The reasons for such losses are unknown, but it is generally believed that “superfluous” metabolic activities were lost by chance during evolution (3). We examined the dependence of the fly *Drosophila pachea* on the senita cactus (*Lophocereus schottii*), a plant species endemic to the Sonoran desert (northwestern Mexico and southwestern United States). In insects, developmental transitions and egg production are regulated by the steroid hormone ecdysone (4).

However, *D. pachea* has lost the first metabolic reaction in the ecdysone biosynthetic pathway, i.e., the ability to convert cholesterol into 7-dehydrocholesterol (7DHC) (Fig. 1A) (4–7). The senita cactus, which *D. pachea* requires as a host (5), does not contain common sterols and is the only plant in the Sonoran desert (7) known to produce $\Delta 7$ -sterols such as lathosterol (6). *D. pachea* flies do not reach the adult stage if not raised on senita cactus, but supplementing standard food with senita cactus or with 7DHC fully restores *D. pachea* viability and fertility (5), indicating that $\Delta 7$ -sterols are essential compounds required for *D. pachea* development and survival.

Interestingly, *D. pachea* appears to depend on the senita cactus solely for its sterols, as we raised *D. pachea* on an artificial diet supplemented with 7DHC for more than 4 years (~60 generations) with no apparent defect (8).

Conversion of cholesterol into 7DHC is catalyzed by the evolutionarily conserved Rieske-domain oxygenase Neverland (NVD) in insects and nematodes (9, 10). To investigate whether mutation(s) in *nvd* are responsible for *D. pachea* dependence on its host cactus, we sequenced the *nvd* coding region (8) from *D. pachea* and the three most closely related species—*D. nanoptera*, *D. acanthoptera*, and *D. wassermani*—which feed on other cacti (11) (tables S1 and S2 and fig. S1). No stop codon or insertions/deletions were found in the *D. pachea* sequence, but the ratio of rates of nonsynonymous substitution (d_N) over synonymous substitution (d_S) is significantly higher in the branch leading to *D. pachea* (table S3 and fig. S2). We noticed that several amino acids showing high conservation across insects and vertebrates are different in *D. pachea* NVD (Fig. 1, B and C).

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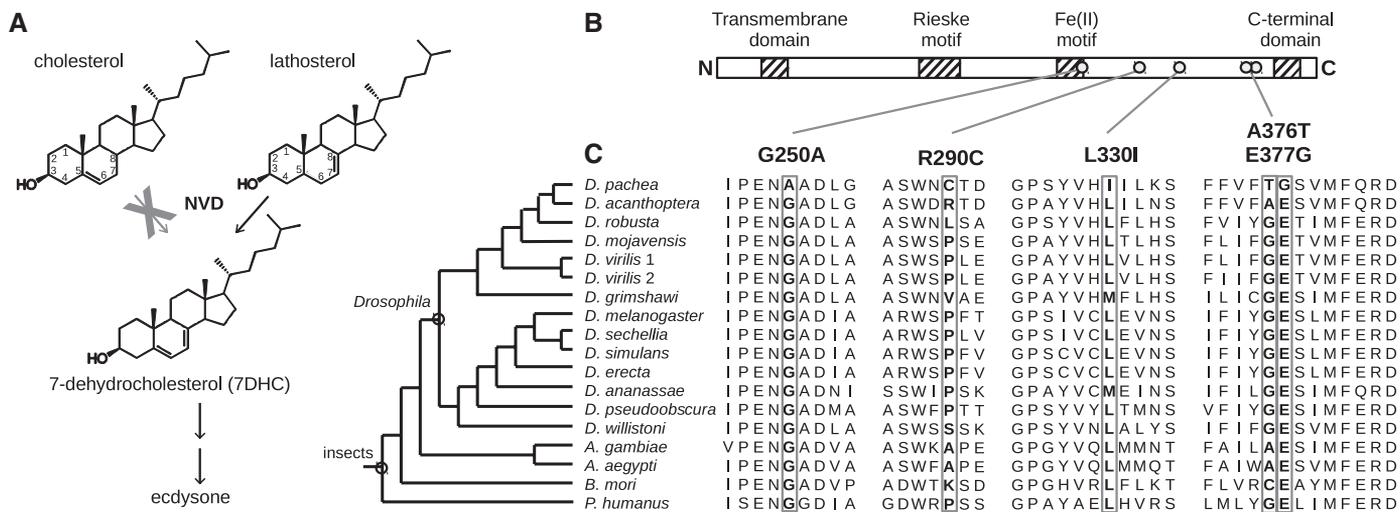


Fig. 1. Presumed ecdysone biosynthetic pathway (A) and NVD in *D. pachea*. (B) NVD protein structure. (C) Alignment of multiple NVD protein sequences. Five mutations (boxes) were tested in vitro. For an alignment of full NVD protein sequences with additional insect species, see fig. S8.

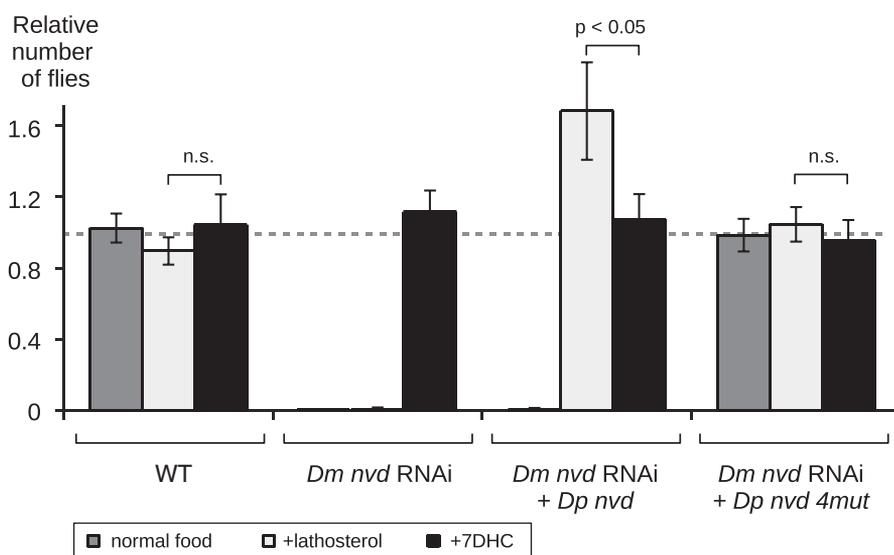


Fig. 2. Fly survival on various food media. WT, control flies; *Dm nvd* RNAi, RNAi knockout of WT *D. melanogaster nvd*; +*Dp nvd*, rescued with WT *D. pachea nvd*; +*Dp nvd* 4mut, rescued with *D. pachea nvd* A250G I330L T376A G377E. The proportion of flies of each genotype is indicated relative to the number of *UAS-nvd* RNAi *Sb* male siblings (8) (table S4). Bars show average, and error bars are mean ± SE.

We observed that in *D. pachea* third instar larvae, as in *D. melanogaster* (9) and *D. acanthoptera*, *nvd* is only expressed in the prothoracic gland (fig. S3), an organ whose sole known function is ecdysone production (12). Therefore, we conclude that NVD function, if any, should be related to steroid hormone production.

The senita cactus does not contain cholesterol or 7DHC but does produce three other sterols—lathosterol, campestenol, and schottenol (6)—that, if used as precursors for steroid hormone synthesis, are expected to lead to different steroid hormones—respectively, 20-hydroxyecdysone, makisterone A, and makisterone C (fig. S4)—due to the inability of *Drosophila* to dealkylate phytosterols (13). Steroids from *D. pachea* ex-

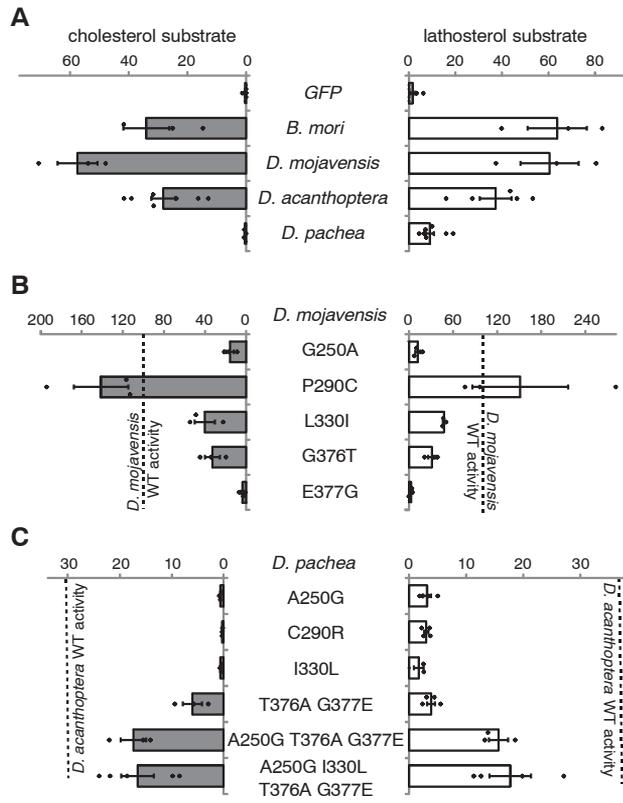
tracts were separated by high-performance liquid chromatography, and fractions of interest were analyzed with mass spectroscopy. We detected ecdysone and 20-hydroxyecdysone but no trace of makisterone A or makisterone C (fig. S5). These results suggest that *D. pachea* only uses lathosterol and not the other senita cactus sterols as steroid hormone precursors.

Because conversion of cholesterol into 7DHC biochemically resembles the transformation of lathosterol into 7DHC (Fig. 1A), we hypothesized that *D. pachea* NVD converts lathosterol rather than cholesterol into 7DHC (14). To test this hypothesis, we generated transgenic *D. melanogaster* flies in which the endogenous *nvd* gene is shut down by RNA interference (RNAi)

and replaced by *D. pachea nvd*. The *D. melanogaster nvd* RNAi flies do not develop on regular fly food (9) or on food supplemented with lathosterol, yet they reach the adult stage on regular fly food supplemented with 7DHC (9) (Fig. 2 and table S4). As expected, introduction of *D. pachea nvd* into *D. melanogaster nvd* RNAi flies rescues development on food supplemented with lathosterol but not with cholesterol (Fig. 2). This demonstrates that *D. pachea* NVD can use lathosterol, but not cholesterol, as a substrate (Fig. 1A).

To identify the amino acid changes responsible for the loss of *D. pachea* NVD activity with cholesterol, we reconstructed ancestral NVD sequences (8) for the entire protein region except for the N-terminal region, which does not show conserved amino acid sequence among insects. Interestingly, we found 19 mutations in the lineage leading to *D. pachea*, of which five are predicted to affect protein function (Fig. 1C). We sequenced the entire *nvd* coding region in three *D. pachea* strains and in two natural population samples. The five predicted functionally relevant amino acids were found in all 32 individuals. To test whether these five amino acid changes affect NVD activity, we established an in vitro assay of NVD activity with green fluorescent protein (GFP)—control and NVD constructs (8, 15). GFP-transfected cells produced no 7DHC, whereas cells transfected with *nvd* from various insects, including *D. acanthoptera*, converted cholesterol and lathosterol into 7DHC (Fig. 3A and fig. S6). In accordance with our *D. melanogaster* transgenic assays, we observed that cells transfected with *D. pachea nvd* do not convert cholesterol into 7DHC but do convert lathosterol into 7DHC (Fig. 3A and fig. S6), although at a lower level relative to other species. Control experiments with hemagglutinin epitope-tagged NVD constructs revealed that *D. pachea* NVD accumulates at similar levels as the other NVD homologs in the in vitro assay (fig. S7). These results indicate that

Fig. 3. NVD enzyme activity with cholesterol (left, gray) or with lathosterol (right, white). **(A)** WT NVD enzymes. **(B)** *D. mojavensis* NVD enzymes containing single mutations. **(C)** *D. pachea* enzymes containing reverse mutations. Bars represent average activity, error bars mean \pm SD, and dots data points. Two *D. mojavensis* *nvd* WT constructs were used in our assays. Enzyme activity is indicated as a percentage relative to the NVD activity obtained with a *D. mojavensis* *nvd* construct that includes the *nvd* gene 5' untranslated region (5'UTR) (9). All the *D. mojavensis* constructs tested in (B) contained this 5'UTR. The dotted line indicates *D. mojavensis* NVD WT activity (construct containing the 5'UTR) in (B) and *D. acanthoptera* NVD WT activity in (C).



the ancestral *Drosophila* NVD enzyme was likely able to transform both cholesterol and lathosterol into 7DHC and that NVD has subsequently lost the ability to convert cholesterol in the lineage leading to *D. pachea*.

We tested the effect of the five predicted functionally relevant amino acid changes by introducing each mutation individually in the *nvd* sequence from *D. mojavensis*, another cactophilic species endemic to the Sonoran desert, which displayed the highest in vitro NVD activity. With either cholesterol or lathosterol as a substrate, substitution P290C slightly increased the activity, G376T and L330I decreased the activity by half, and substitutions of G250A and E377G reduced the activity to less than 18% of the wild-type (WT) activity (Fig. 3B and fig. S7). We also performed the reciprocal experiment and reintroduced the predicted ancestral amino acid residues into the *D. pachea* NVD sequence. We found that NVD activity close to that of *D. acanthoptera* is not restored by a single amino acid change but by four amino acid changes in concert (Fig. 3C and fig. S7). Corroborating these in vitro results, introduction of a *D. pachea* *nvd* construct containing these four amino acid changes into *D. melanogaster* *nvd* RNAi flies rescues development on food supplemented with cholesterol (Fig. 2). We conclude that two to four mutations

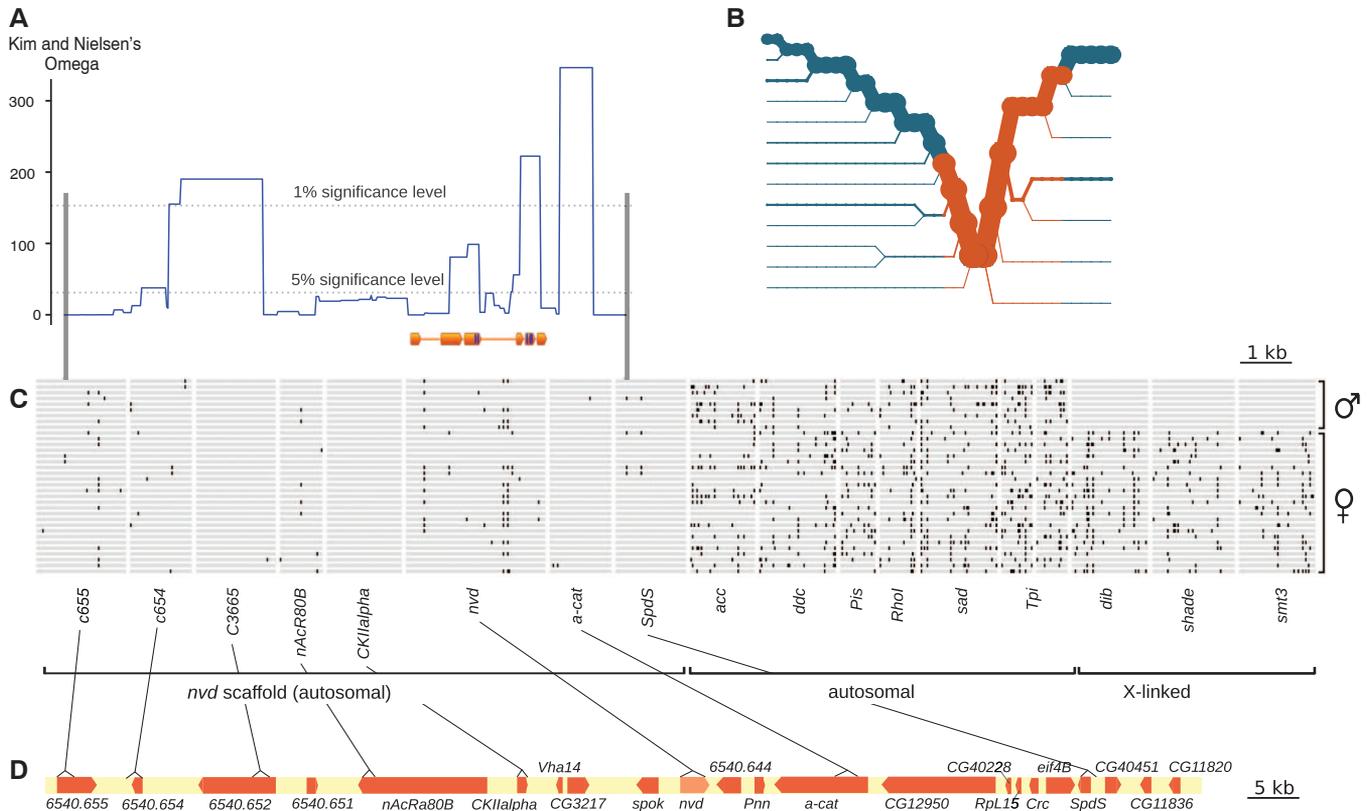


Fig. 4. The *nvd* region is under positive selection. **(A)** Kim and Nielsen's omega statistic (17) across the *nvd* region. Omega values above the significance level indicate a selective sweep. The *nvd* coding regions are represented below, with the position of the five tested amino acid changes in purple. **(B)** Haplotype bifurcation plot. Circles indicate polymorphic sites in the *nvd* gene (orange) and in neigh-

boring loci (blue). Line thickness is proportional to the number of samples with the indicated haplotype. **(C)** Representation of the genotypes of 34 individuals. Black bars indicate heterozygote positions. Homozygote sites for rare alleles are not shown. **(D)** Position of the sequenced loci within the *nvd* region. Gene annotations are in orange.

in the *D. pachea nvd* coding region have caused the loss of NVD activity with cholesterol substrate. These mutations have turned *D. pachea* into an obligate specialist dependent on lathosterol, a compound that has been found in a single plant species in the Sonoran desert (5, 6).

Remarkably, *D. melanogaster nvd* RNAi flies expressing *D. pachea nvd* survive significantly better on lathosterol than on cholesterol (*t* test, $t_{10,11} = 2.029$, $P < 0.03$) (Fig. 2), but no effect on survival was detected with *nvd* RNAi flies expressing *D. pachea nvd* with the four ancestral amino acid changes (Fig. 2). This suggests that the mutations that abolished cholesterol conversion during *D. pachea* evolution provide a fitness advantage on lathosterol. The underlying mechanism remains unclear. Our in vitro assay does not uncover any benefit from the *D. pachea nvd* mutations: *D. pachea* NVD in vitro activity with lathosterol is not higher compared with other species (Fig. 3), and the NVD enzymes of related *Drosophila* species are already able to convert lathosterol into 7DHC. To assess population genetic forces at play on the *nvd* genomic region, we compared the 3-kb *nvd* locus and seven genes on the same 100-kb scaffold with nine control genes in 34 individuals from a single natural population. Our analysis reveals that *nvd* is in a genomic region of low nucleotide diversity, low recombination rate, and normal divergence rate (McDonald-Kreitman test, $P > 0.85$; maximum likelihood extension of the Hudson-Kreitman-Aguadé test, $P < 10^{-5}$) (Fig. 4 and tables S5 to S11). A signature of a selective sweep is detected [Kim and Nielsen omega (17)] over *nvd* and neighboring loci (Fig. 4), but nucleotide polymorphism is too low to infer whether this recent selection acted on the *nvd* mutations themselves. Tajima's *D* and Fu and Li tests are consistent with recovery from selective sweep in the *nvd* region (table S6).

A likely scenario is that *D. pachea* first evolved a resistance toward senita cactus toxic compounds (5) and slowly became restricted to this food source as it escaped competition with other fly species. Evolution of *D. pachea*'s resistance most likely did not involve NVD because *nvd* is not expressed in the midgut and fat body (fig. S3), the detoxification organs in insects (16). As lathosterol became *D. pachea*'s unique source of sterols for steroid hormone synthesis, mutations in *nvd* that abolished NVD activity on cholesterol appeared and were fixed rapidly due to their beneficial effect with lathosterol. As a result, *D. pachea* became an obligate specialist on the senita cactus. We point out that besides *nvd* mutations, mutation(s) in other genes might also have contributed to *D. pachea* dependence on lathosterol. Alternatively, the identified *nvd* mutations may have spread while *D. pachea* ancestors were still feeding on various plants and may thus have accelerated its ecological specialization. Our study, which uncovered several mutations underlying the obligate bond between a specialist species and its host, illustrates how a

few mutations in a single gene can restrict the ecological niche of a species.

References and Notes

1. A. Lwoff, *L'Évolution physiologique: Étude des pertes de fonctions chez les microorganismes* (Hermann, Paris, 1944).
2. P. Romero *et al.*, *Genome Biol.* **6**, R2 (2005).
3. D. Voet, *Biochemistry* (Wiley, Somerset, 1995).
4. X. Huang, J. T. Warren, L. I. Gilbert, *J. Genet. Genomics* **35**, 1 (2008).
5. W. B. Heed, H. W. Kircher, *Science* **149**, 758 (1965).
6. C. E. Campbell, H. W. Kircher, *Phytochemistry* **19**, 2777 (1980).
7. J. C. Fogleman, P. B. Danielson, *Am. Zool.* **41**, 877 (2001).
8. Materials and methods are available as supplementary materials on Science Online.
9. T. Yoshiyama, T. Namiki, K. Mita, H. Kataoka, R. Niwa, *Development* **133**, 2565 (2006).
10. V. Rottiers *et al.*, *Dev. Cell* **10**, 473 (2006).
11. S. Pitnick, W. B. Heed, *Ann. Entomol. Soc. Am.* **87**, 307 (1994).
12. R. Lafont, C. Dauphin-Villemant, J. T. Warren, H. H. Rees, in *Comprehensive Molecular Insect Science*, L. I. Gilbert, K. Iatrou, S. Gill, Eds. (Oxford, 2005), vol. 3, pp. 125–195.
13. C. Blais, T. Blasco, A. Maria, C. Dauphin-Villemant, R. Lafont, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **878**, 925 (2010).
14. K. C. Goodnight, H. W. Kircher, *Lipids* **6**, 166 (1971).
15. T. Yoshiyama-Yanagawa *et al.*, *J. Biol. Chem.* **286**, 25756 (2011).
16. O. Mittapalli *et al.*, *PLoS ONE* **5**, e13708 (2010).
17. Y. Kim, R. Nielsen, *Genetics* **167**, 1513 (2004).

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Supplementary Materials

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Fermentation, Hydrogen, and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla

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BD1-5, OP11, and OD1 bacteria have been widely detected in anaerobic environments, but their metabolisms remain unclear owing to lack of cultivated representatives and minimal genomic sampling. We uncovered metabolic characteristics for members of these phyla, and a new lineage, PER, via cultivation-independent recovery of 49 partial to near-complete genomes from an acetate-amended aquifer. All organisms were nonrespiring anaerobes predicted to ferment. Three augment fermentation with archaeal-like hybrid type II/III ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) that couples adenosine monophosphate salvage with CO₂ fixation, a pathway not previously described in Bacteria. Members of OD1 reduce sulfur and may pump protons using archaeal-type hydrogenases. For six organisms, the UGA stop codon is translated as tryptophan. All bacteria studied here may play previously unrecognized roles in hydrogen production, sulfur cycling, and fermentation of refractory sedimentary carbon.

Sequencing of total DNA recovered directly from natural systems (metagenomics) often reveals previously unknown genes (1, 2)

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and has the potential to yield near-complete genomes suitable for metabolic and phylogenetic analyses (3–5). Numerous bacteria are known exclusively through cultivation-independent recovery of their ribosomal RNA (rRNA) genes and thus are important targets for this approach (6). Here, we sequenced DNA from three microbial communities from an acetate-amended aquifer to reconstruct genomes of organisms that may contribute to biogeochemical cycling in anoxic