Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids

Nancy A. Moran, *et al.*

Science **328**, 624 (2010);
DOI: 10.1126/science.1187113

Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids

Nancy A. Moran[1]* and Tyler Jarvik[2]

Carotenoids are colored compounds produced by plants, fungi, and microorganisms and are required in the diet of most animals for oxidation control or light detection. Pea aphids display a red-green color polymorphism, which influences their susceptibility to natural enemies, and the carotenoid torulene occurs only in red individuals. Unexpectedly, we found that the aphid genome itself encodes multiple enzymes for carotenoid biosynthesis. Phylogenetic analyses show that these aphid genes are derived from fungal genes, which have been integrated into the genome and duplicated. Red individuals have a 30-kilobase region, encoding a single carotenoid desaturase that is absent from green individuals. A mutation causing an amino acid replacement in this desaturase results in loss of torulene and of red body color. Thus, aphids are animals that make their own carotenoids.

Carotenoids are a distinctive, widespread class of molecules with diverse metabolic and ecological roles in organisms (*1*). Variants of these colored compounds are synthesized with the same small set of homologous enzymes, of which copies are distributed in many species of Bacteria, Archaea, Fungi, and plants. Animals require carotenoids for several functions, ranging from ornamentation to antioxidants and immune system modulators to precursors for visual pigments [e.g., (*1*–*4*)]. But animals obtain these compounds from food, and so far, no animal has been reported to make its own carotenoids. Here we report the presence and expression of carotenoid biosynthetic genes in aphids (Insecta: Hemiptera). Further, we show that they underlie production of carotenoids and color, including a genetic color polymorphism affecting interactions with natural enemies (*5*). Phylogenetic analyses imply the ancestral transfer of these genes from a fungus to an ancestor of numerous modern aphid species.

Carotenoids have been reported from several species of aphids, and carotenoid content has been shown to differ between color morphs in two color polymorphic species, *Macrosiphum liriodendron* and *Sitobion avenae* (*6*–*10*). Green forms contain α-, β-, and γ-carotene (all yellow or yellow-orange compounds), whereas red (or brownish) forms of the same species also contain lycopene or torulene (red compounds) (*6*, *7*, *10*).

The pea aphid, *Acyrthosiphon pisum*, displays a red-green color polymorphism (Fig. 1, A and B) in which color is stable within all-female parthenogenetic clones (although environmental factors can cause temporary variation within each type). The color polymorphism appears to be maintained by frequency-dependent selection imposed by natural enemies that search for prey using different visual cues, which results in differential susceptibility of the red and green individuals (*5*, *11*).

Carotenoid assays of *A. pisum* samples from our laboratory colonies (*12*) revealed that green clones contain mostly γ-carotene, β-carotene, and α-carotene, whereas red clones contain these compounds plus torulene and dehydro-γ,ψ-carotene (a carotenoid similar to torulene) (Fig. 1D). These two compounds, completely absent from green clones (Fig. 1D), can be derived from γ-carotene through a desaturation step (fig. S1) (*13*, *14*). They are vermilion (bright red), the color of the water-insoluble pigments extracted from red *A. pisum*, whereas the other carotenoids are pale to bright yellow, the color of the water-insoluble pigments extracted from green *A. pisum*.

Because animals are generally considered to lack the enzymatic machinery required for carotenoid biosynthesis, one explanation for the presence of carotenoids in aphids is that they are sequestered from the diet. However, carotenoids, as lipid-soluble compounds, are not expected to occur in significant quantities in phloem sap; furthermore, the carotenoid profiles of aphids differ dramatically from those in their host plants (*8*). An alternative explanation, proposed by several authors, is that aphids acquire carotenoids from their bacterial endosymbionts (*7*, *9*, *10*). However, genome sequencing has revealed that neither the primary symbiont *Buchnera aphidicola* (*15*) nor two facultative symbionts (*16*, *17*) have any genes with homology to carotenoid biosynthetic genes. Furthermore, facultative endosymbionts can be eliminated from clones or transferred between clones without affecting color (*18*, *19*). Finally, such endosymbionts are inherited maternally, whereas, in both *A. pisum* and the peach-potato aphid, *Myzus persicae*, red-green color shows Mendelian inheritance, with red dominant to green in both species (*20*–*22*). We verified this pattern of inheritance in our laboratory lines of *A. pisum* (table S1). Thus, these colors are dependent on genes encoded in the aphid genome. In principle, such genes could affect the ability to sequester or display carotenoids rather than encoding enzymes of carotenoid biosynthesis directly.

The recent release of the genome sequence of *A. pisum* (*23*) allowed us to search for carotenoid

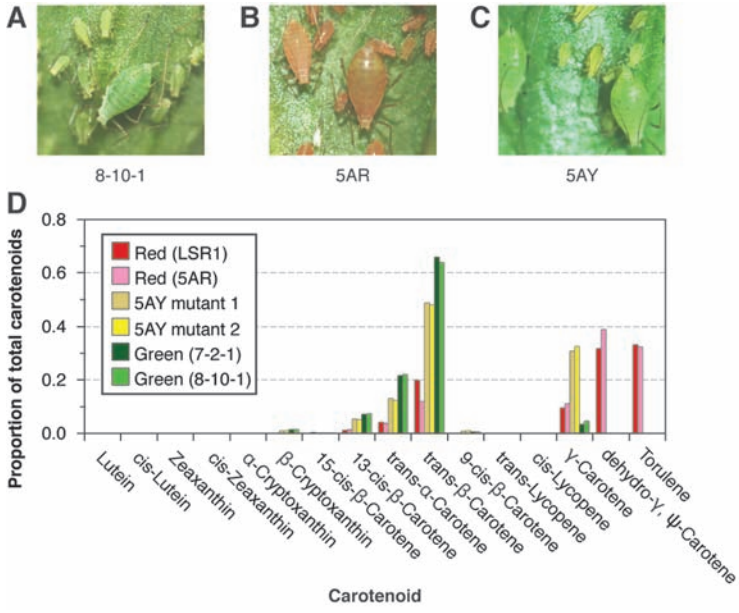

Fig. 1. Coloration and carotenoids in the pea aphid. Typical green (**A**) and red (**B**) aphid clones, (**C**) 5AY, a green mutant clone arising from the red clone 5A. (**D**) Profiles of carotenoids in red (5A, LSR1), mutant red→green (5AY, two samples), and green (8-10-1, 7-2-1) pea aphid clones. Torulene and a related red compound are restricted to red clones; the mutant 5AY clone lacks these and displays an elevation in their predicted precursor, γ-carotene.

[1]Department of Ecology and Evolutionary Biology, 1041 East Lowell Street, University of Arizona, Tucson, AZ 85721, USA. [2]Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA.

*To whom correspondence should be addressed. E-mail: nancy.moran@yale.edu

biosynthetic genes. Searches against a database of RefSeq proteins inferred from the genome, using as queries sequences of carotenoid biosynthetic enzymes from bacteria and plants, revealed close homology with four carotenoid desaturase homologs and three proteins consisting of fused carotenoid cyclase–carotenoid synthase enzymes (Table 1). Searching the GenBank protein database using the inferred aphid proteins as queries revealed the closest sequence homology to carotenoid biosynthetic genes of several fungi, but no detectable homology to enzymes encoded by any other available animal genome. In phylogenetic analyses for both the carotenoid cyclase–carotenoid synthase proteins and for the carotenoid desaturase proteins, the aphid copies form a highly supported clade that is nested within a fungal clade with strong support (Fig. 2, A and B). Furthermore, the relation to fungal enzymes is strongly supported by the similarity of the gene arrangement between *A. pisum* and certain fungi. Although bacteria and plants also contain homologs of these genes, only fungi display a fusion of carotenoid cyclase and carotenoid synthase (*24*–*26*). Furthermore, in *A. pisum*, all three carotenoid synthase–carotenoid cyclase and three of the four carotenoid desaturase genes are paired with divergent orientation of transcription in aphid genome scaffolds; this arrangement is otherwise only described from certain fungi (fig. S2) (*25*, *26*).

Contamination of the DNA sample used for the aphid genome project with fungal DNA was ruled out as an explanation for the presence of these genes. In the *A. pisum* genome project, all of the carotenoid biosynthetic genes occur on large scaffolds that contain other genes with closest homology to other insects or repetitive elements characteristic of the aphid genome. Coding regions of carotenoid genes show typical depth of coverage and are joined to aphid-specific sequences on individual clone inserts. Furthermore, expression of all seven *A. pisum* genes was supported by expressed sequence tag (EST) data collected from aphids grown in several laboratories (Table 1). Polymerase chain reaction (PCR) experiments confirmed the presence and expression of these genes in all tested samples of *A. pisum,* both from field collections and from laboratory colonies. The only exception was one carotenoid desaturase locus, which was absent from green clones of pea aphids, as explained further below.

Taken together, this evidence supports the transfer of these genes from a fungus to an aphid ancestor as a single event, followed by duplication within the aphid genome. Such transfer preserved the gene arrangement observed in certain fungi, in which the entire region, encompassing divergently transcribed carotenoid desaturase and carotenoid synthase–carotenoid cyclase loci, comprises only about 5 kilobases (kb) (*25*, *26*). The aphid copies have much larger introns and larger intergenic spacers (Table 1 and fig. S2), which reflect typical gene structure in the aphid genome (*23*). The gene arrangement differs from that in studied species of Ascomycetes (*27*), which reinforces our phylogenetic evidence that the donor was not in this group. Potentially, the ancestral gene donor was a fungal pathogen or symbiont of aphids, or of an aphid host plant.

In both the carotenoid cyclase and carotenoid desaturase trees, *A. pisum* sequences and EST sequences from *Myzus persicae* form clades together, which implies that the transfer preceded their shared ancestor, at the base of the aphid clade corresponding to the aphid tribe Macrosiphini.

We used the red-green genetic polymorphism and a spontaneously arising laboratory mutant (Fig. 1, A to C) to determine whether a difference in one of these loci underlies observed differences between *A. pisum* clones in color and in carotenoid content. First, we sequenced the full-length genomic DNA containing these genes for two green clones (8-10-1 and SCC13), two red clones (5A and LSR1), and two samples of a mutant yellow-green clone that arose from clone 5A but lacked red color (clone 5AY). Most genes gave highly similar products for all lines, with a low level of allelic divergence, which indicated heterozygosity of about 0.13%, generally consistent with previous studies of sequence variation in *A. pisum* populations (*28*). However, both green lines failed to amplify for any primer pairs designed in the region of one of the four copies of carotenoid desaturase (XP_001943938, corresponding to a portion of Scaffold NW_001918682, Table 1), whereas both red lines gave products and sequences that were identical (LSR1) or near identical (5A) to the sequence from the genome project. (LSR1 was used in the genome project.) Furthermore, both sequenced red lines showed no heterozygosity in this region in contrast to all other regions, which showed clear evidence of heterozygosity. After manual assembly of traces from the genome project and from our own sequencing, we reconstructed a 30-kb scaffold for this region, which we resequenced for LSR1 (fig. S3).

These observations suggest that the red lines are heterozygous and that the red allele contains over 30 kb missing in the green allele. Genetic crosses confirm that both of these red lines were heterozygous for color, giving rise to a mixture of red and green progeny when crossed with green lines (table S1). In PCR screens of 60 F_1 lines from the largest such cross, all red progeny yielded amplicons of the expected length, whereas no green

Table 1. Genes in the *A. pisum* genome with closest homology to carotenoid biosynthetic enzymes, including scaffold of origin and matching EST sequences. Similar color indicates that the gene is on the same scaffold. The 3′ end of scaffold NW_001925130 overlaps with the 5′ end of NW_001923501 for 5400 base pairs, and PCR demonstrated continuity of these scaffolds. Pink row is the gene corresponding to *tor*^{R} and conferring red color (see text). Protein length, amino acids; ESTs are those present in GenBank, mostly from clone LSR1.

Enzyme type	Protein		mRNA		ESTs	LOC_ID/ ACYPI_ID	Scaffold		Gene		
	Accession	Length (aa)	Accession	Length (bp)	(*n*)		Accessions	Length (bp)	Start	End	Length (bp)
Carotenoid synthase/ cyclase	XP_001943170	608	XM_001943135	1,981	4	LOC100161104/ ACYPI002354	NW_001938125/ SCAFFOLD9039	96,434	60,929	75,839	14,910
Carotenoid synthase/ cyclase	XP_001950787	588	XM_001950729	2,223	9	LOC100159332/ ACYPI000715	NW_001925130/ SCAFFOLD17863	49,317	1,564	8,266	6,702
Carotenoid synthase/ cyclase	XP_001950868	589	XM_001950833	1,770	8	LOC100164140/ ACYPI005179	NW_001925130/ SCAFFOLD17863	49,317	36,365	42,379	6,014
Carotenoid desaturase	XP_001943225	373	XM_001943190	1,247	3	LOC100159050/ ACYPI000460	NW_001938125/ SCAFFOLD9039	96,434	76,368	93,106	16738
Carotenoid desaturase	XP_001950764	528	XM_001950729	2,718	7	LOC100161380/ ACYPI002604	NW_001925130/ SCAFFOLD17863	49,317	19,665	22,451	2,786
Carotenoid desaturase	XP_001946689	526	XM_001946654	2,693	25	LOC100169110/ ACYPI009757	NW_001923501/ SCAFFOLD16397	29,128	8,574	16,388	7,814
Carotenoid desaturase	XP_001943938	510	XM_001943903	2,410	57	LOC100169245/ ACYPI009883	NW_001918682/ SCAFFOLD12059	31,283	1,415	8,641	7,226

progeny yielded amplicons for sequences within this region. To determine whether the correlation of this genomic region with color extended across green and red clones generally, we surveyed several green and red *A. pisum* clones from North American locations. This region was present in every red aphid sample and absent from all green aphid samples (fig. S4).

We designated this locus *tor* (for torulene production), with alleles tor^R and tor^G. The structure of tor^G was not determined but is inferred to consist of a large deletion relative to tor^R. Males derived from a heterozygous clone (5A) all have tor^R, which indicates that it occurs on an autosome and not the X chromosome, for which males are haploid.

The mutant line 5AY has stable yellow-green color (Fig. 1C) and has a carotenoid content similar to green clones except that γ-carotene is elevated relative to β- and α-carotene (Fig. 1D). In contrast to the red parental line (5A), 5AY also resembles green clones in completely lacking torulene and dehydro-γ,ψ-carotene (Fig. 1D). Genomic sequences corresponding to coding regions for all seven carotenoid biosynthetic genes were obtained for 5AY. All sequences were identical to those of 5A for a total of 60 kilobase pairs of sequence, except for a single base difference within the tor^R allele. This mutation was derived in the 5AY lineage in the laboratory. This G→A substitution was predicted to cause a single–amino acid replacement (glutamic acid→lysine at position 32). This replacement affects the substrate-binding site of the carotenoid desaturase and results in a change from a negatively charged to a positively charged residue at a site that is conserved across members of this family from bacteria, plants, and fungi (fig. S5). This mutation therefore appears to be a radical change in the *tor* locus that results in a failure to make torulene and dehydro-γ,ψ-carotene and the ensuing accumulation of the predicted substrate, γ-carotene.

Experiments in bacteria and fungi show that distinct carotenoid profiles can result from small changes in enzyme amino acid sequence or expression (*14*, *24*). Thus, following a single transfer of carotenoid biosynthetic genes from a fungus to aphids, gene duplications, sequence diversification, and shifts in expression of copies could have resulted in a variety of carotenoids that contribute to aphid colors and affect color polymorphisms in several species (*6*, *7*, *10*, *20*). In *A. pisum*, the red-green polymorphism has been shown to affect susceptibility to natural enemies (*5*, *11*, *29*), and aphid carotenoids may confer other benefits not yet investigated.

Recent studies in animals have revealed several cases of DNA acquisition from bacterial sources (*30*, *31*), including some cases involving functional genes (*32*–*34*). A comprehensive search of the *A. pisum* genome for bacterium-derived genes revealed a total of only 12 apparently functional transferred genes, derived from a smaller number of acquisition events followed by duplication (*33*). However, that search did not use a strategy designed to identify genes derived from fungi or other eukaryotes, so the carotenoid biosynthetic genes were not detected. We searched the *A. pisum* genome for additional genes derived from fungi but detected only the seven loci homologous with genes for carotenoid biosynthesis (Table 1).

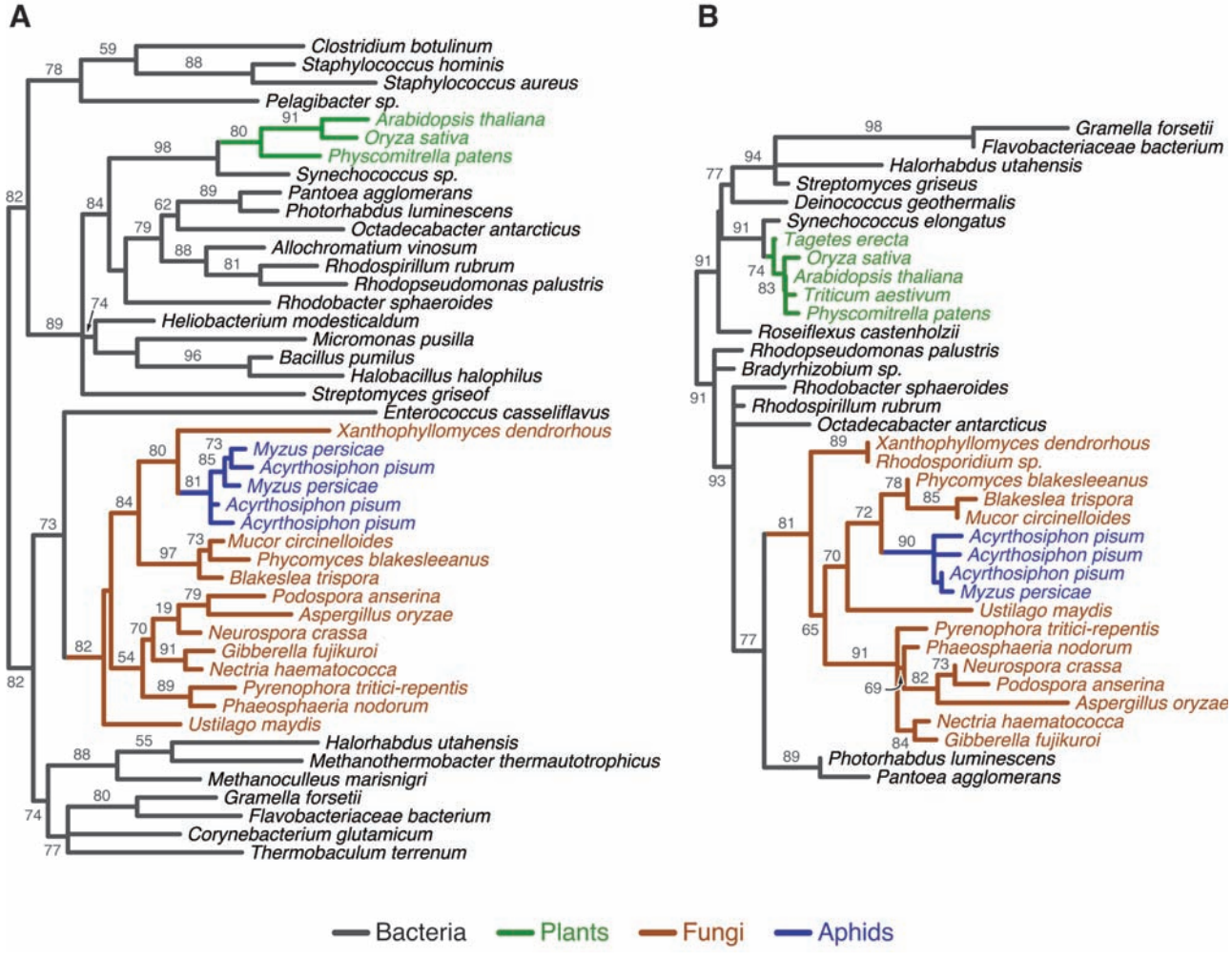

Fig. 2. Phylogenetic relations of inferred carotenoid biosynthetic enzymes from the pea aphid genome. (**A**) Carotenoid desaturases and (**B**) carotenoid cyclase–carotenoid synthases. Sequences are from aphids, bacteria, plants, and fungi; no homologs were detectable in other sequenced animal genomes. Bootstrap support greater than 50% is indicated on branches.

Our case is unusual in that the genes originate from a fungus and have a known ecological role in the recipient. In view of the widespread dependence of animals on carotenoids, it is perhaps curious that acquisition of genes underlying carotenoid biosynthesis has not been more frequent. Whereas the phylogenies for these genes suggest several events of horizontal gene transfer among divergent bacterial lineages (Fig. 2), the trees support only a single acquisition by plants (from their plastid symbionts) and a single origin within Fungi (Fig. 2). Likewise, the transfer documented here, from a fungus to an aphid ancestor, is, so far, the only acquisition of carotenoid biosynthetic machinery known in animals.

References and Notes

1. T. W. Goodwin, *Annu. Rev. Nutr.* **6**, 273 (1986).
2. P. D. Fraser, P. M. Bramley, *Prog. Lipid Res.* **43**, 228 (2004).
3. M. Rothschild, in *Coevolution of Animals and Plants*, L. E. Gilbert, P. H. Raven, Eds. (Univ. of Texas Press, Austin, 1975), pp. 20–47.
4. A. V. Badyaev, *Evol. Biol.* **34**, 61 (2007).
5. J. E. Losey, A. R. Ives, J. Harmon, F. Ballantyne, C. Brown, *Nature* **388**, 269 (1997).
6. A. G. Andrewes *et al.*, *Acta Chem. Scand.* **25**, 3878 (1971).
7. K. S. Brown, *Chem. Soc. Rev.* **4**, 263 (1975).
8. B. Czeczuga, *Zool. Pol.* **25**, 27 (1976).
9. E. J. Houk, *J. Insect Physiol.* **20**, 471 (1974).
10. R. L. Jenkins, H. D. Loxdale, C. P. Brookes, A. F. G. Dixon, *Physiol. Entomol.* **24**, 171 (1999).
11. J. P. Harmon, J. E. Losey, A. R. Ives, *Oecology* **115**, 287 (1998).
12. Materials and methods are available as supporting material on *Science* Online.
13. J. C. Verdoes *et al.*, *Appl. Environ. Microbiol.* **69**, 3728 (2003).
14. D. Umeno, A. V. Tobias, F. H. Arnold, *Microbiol. Mol. Biol. Rev.* **69**, 51 (2005).
15. S. Shigenobu, H. Watanabe, M. Hattori, Y. Sakaki, H. Ishikawa, *Nature* **407**, 81 (2000).
16. P. H. Degnan, Y. Yu, N. Sisneros, R. A. Wing, N. A. Moran, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 9063 (2009).
17. P. H. Degnan *et al.*, *Environ. Microbiol.*, published online 16 October 2009 (www3.interscience.wiley.com/cgi-bin/fulltext/122652702/HTMLSTART.
18. K. M. Oliver, J. A. Russell, N. A. Moran, M. S. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1803 (2003).
19. K. M. Oliver, N. A. Moran, M. S. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12795 (2005).
20. F. P. Müller, *Archiv Freunde Naturgesch. Mecklenburg* **7**, 228 (1961).
21. F. P. Müller, *Z. Pflanzenkr.* **69**, 129 (1962).
22. H. Takada, *Appl. Entomol. Zool. (Jpn.)* **16**, 242 (1981).
23. International Aphid Genomics Consortium, *PLoS Biol.* **8**, e1000313 (2010).
24. J. C. Verdoes, K. P. Krubasik, G. Sandmann, A. J. van Ooyen, *Mol. Gen. Genet.* **262**, 452 (1999).
25. N. Arrach, R. Fernández-Martín, E. Cerdá-Olmedo, J. Avalos, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1687 (2001).
26. M. Rodríguez-Sáiz *et al.*, *Appl. Environ. Microbiol.* **70**, 5589 (2004).
27. P. Linnemannstöns, M. M. Prado, R. Fernández-Martín, B. Tudzynski, J. Avalos, *Mol. Gen. Genet.* **267**, 593 (2002).
28. J. A. Brisson, S. V. Nuzhdin, D. L. Stern, *BMC Genet.* **10**, 22 (2009).
29. R. Libbrecht, D. M. Gwynn, M. D. E. Fellowes, *J. Insect Behav.* **20**, 25 (2007).
30. N. Kondo, N. Nikoh, N. Ijichi, M. Shimada, T. Fukatsu, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14280 (2002).
31. J. C. Hotopp *et al.*, *Science* **317**, 1753 (2007).
32. N. Nikoh, A. Nakabachi, *BMC Biol.* **7**, 12 (2009).
33. N. Nikoh *et al.*, *PLoS Genet.* **6**, e1000827 (2010).
34. M. E. Rumpho *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17867 (2008).

35. We are indebted to K. Vogel for samples of aphids, including crosses he performed for other experiments. We thank N. Craft and staff at Craft Technologies for high-performance liquid chromatography analyses of aphid samples, K. Hammond for care of pea aphid colonies, K. Hammond and H. Dunbar for noticing the mutant 5AY female, J. Russell and S. Via for aphid clones, B. Nankivell for preparation of figures and of the manuscript, H. Ochman for comments on the paper, members of the Ochman-Moran laboratory for comments on the project, and A. Badyaev for discussions of carotenoids in animals. This project was funded by NSF 0626716 to N.M. GenBank accession for the DNA sequence is GU456379.

Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5978/624/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
References

15 January 2010; accepted 1 March 2010
10.1126/science.1187113

D-Amino Acids Trigger Biofilm Disassembly

Ilana Kolodkin-Gal,[1] Diego Romero,[2] Shugeng Cao,[3] Jon Clardy,[3] Roberto Kolter,[2] Richard Losick[1]*

Bacteria form communities known as biofilms, which disassemble over time. In our studies outlined here, we found that, before biofilm disassembly, *Bacillus subtilis* produced a factor that prevented biofilm formation and could break down existing biofilms. The factor was shown to be a mixture of D-leucine, D-methionine, D-tyrosine, and D-tryptophan that could act at nanomolar concentrations. D-Amino acid treatment caused the release of amyloid fibers that linked cells in the biofilm together. Mutants able to form biofilms in the presence of D-Amino acids contained alterations in a protein (YqxM) required for the formation and anchoring of the fibers to the cell. D-Amino acids also prevented biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. D-amino acids are produced by many bacteria and, thus, may be a widespread signal for biofilm disassembly.

Most bacteria form multicellular communities known as biofilms in which cells are protected from environmental insults (*1*, *2*). However, as biofilms age, nutrients become limiting, waste products accumulate, and it is advantageous for the biofilm-associated bacteria to return to a planktonic existence (*2*). Thus, biofilms have a finite lifetime, characterized by eventual disassembly. *Bacillus subtilis* forms communities on semi-solid surfaces and thick pellicles at the air/liquid interface of standing cultures (*1*, *3*–*5*). Cells in the biofilms are held together by an extracellular matrix consisting of exopolysaccharide and amyloid fibers composed of the protein TasA (*5*–*7*). The exopolysaccharide is produced by the *epsA-O* operon, and the TasA protein is encoded by the *yqxM-sipW-tasA* operon (*8*). After 3 days of incubation in a biofilm-inducing medium, *B. subtilis* formed thick pellicles at the air/liquid interface of standing cultures (Fig. 1A). Upon incubation for an additional 3 to 5 days, however, the pellicles lost their integrity (Fig. 1B). To investigate whether mature biofilms produce a factor that triggers biofilm disassembly, we asked whether a conditioned medium would prevent pellicle formation when added to a fresh medium (*9*). Medium from an 8-day-old culture was applied to a C18 column (Sep Pak, Waters, Milford, MA), and concentrated eluate from the column was added to a freshly inoculated culture. The eluate was sufficient to prevent pellicle formation (Fig. 1C). Concentrated eluate from a 3-day-old culture had little effect on pellicle formation (Fig. 1D). Further purification of the factor was achieved by eluting the cartridge stepwise with methanol. Elution with 40% methanol resulted in a fraction that was active in inhibiting pellicle formation (Fig. 1E), but had little effect on cell growth (fig. S1). The activity was resistant to heating at 100°C for 2 hours and proteinase K treatment (Fig. 1F).

Bacteria produce D-amino acids in stationary phase (*10*). We asked whether the biofilm-inhibiting factor was composed of one or more D-amino acids. Indeed, D-tyrosine, D-leucine, D-tryptophan, and D-methionine were active in inhibiting biofilm formation in a liquid medium, as well as on a solid medium (Fig. 1, G and H, and figs. S2 and S3). In contrast, the corresponding L-isomers and D-isomers of other amino acids (such as D-alanine and D-phenylalanine) were inert in our biofilm-inhibition assay. Next, we determined the minimum concentration needed to prevent biofilm formation. Individual D-amino acids varied in their activity, with D-tyrosine being more effective (3 μM) than D-methionine

[1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. [2]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA. [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: losick@mcb.harvard.edu