signatures in guiding the perception of properties that seem like complex, high-level judgements: such as translucency [5] and gloss [6,7]. The images used by Marlow *et al.* [4] were purposefully kept simple to parametrically explore the image cues they identified. Real objects pose additional challenges: surfaces vary in more than one direction, the bounding contours may not be visible (for example, the ends of the fabric are out of the frame in Figure 1C) and real surfaces typically have changes in both surface pigmentation and depth profile (for example, the plaid pattern on the fabric in Figure 1C), meaning the different types of variation should be parsed by the brain [8].

Nevertheless, Marlow *et al.* [4] have taken us a step closer to formulating the types of image signature that are characteristic of the different physical causes of images. Given the availability of this information, it seems sensible that the brain is attuned to detect it. Moreover, their stimuli provide a useful new tool with which to investigate the neural architecture that extracts shape from shading in the human brain [3]. Previous studies wrestled with the difficulties of controlling for the image differences between three-dimensional shading *versus* non-three-dimensional greyscale contrasting stimuli needed for functional magnetic resonance imaging (fMRI) [9]. The stimuli developed by Marlow *et al.* [4] would provide an ideal means of comprehensively testing shape-from-shading in human fMRI studies using parametrically-controlled stimuli.

Artists have long used *chiaroscuro* — dramatic directional lighting — to enhance the sense of three-dimensional volume in paintings. The Marlow *et al.* [4] paper reveals key image signatures that combine intensity signals with local orientation and curvature to determine the impression of three-dimensional shape from shading. These simple cues appear to underlie human perceptual judgments, and may allow a relatively simple neural processing architecture that could explain the apparent innate ability of newborn chicks to interpret shading patterns as three-dimensional objects when pecking for food [10].

REFERENCES

1. Pliny, The Elder (1991). Natural History, First Edition (London: Penguin Classics).
2. Koenderink, J.J., and van Doorn, A.J. (2003). Shape and shading. In The Visual Neurosciences, L.M. Chalupa, and J.S. Werner, eds. (Cambridge: MIT Press), pp. 1090–1105.
3. Welchman, A.E. (2016). The human brain in depth: how we see in 3D. Annu. Rev. Vis. Sci. *2*, 345–376.
4. Marlow, P.J., Mooney, S.W.J., and Anderson, B.L. (2019). Photogeometric cues to perceived surface shading. Curr. Biol. *29*, 306–311.
5. Marlow, P.J., Kim, J., and Anderson, B.L. (2017). Perception and misperception of surface opacity. Proc. Natl. Acad. Sci. USA *114*, 13840–13845.
6. Marlow, P.J., Kim, J., and Anderson, B.L. (2012). The perception and misperception of specular surface reflectance. Curr. Biol. *22*, 1909–1913.
7. Muryy, A.A., Fleming, R.W., and Welchman, A.E. (2016). 'Proto-rivalry': how the binocular brain identifies gloss. Proc. R. Soc. Lond. B *283*, 20160383.
8. Dövencioğlu, D.N., Welchman, A.E., and Schofield, A.J. (2013). Perceptual learning of second order cues for layer decomposition. Vis. Res. *77*, 1–9.
9. Georgieva, S.S., Todd, J.T., Peeters, R., and Orban, G.A. (2008). The extraction of 3D shape from texture and shading in the human brain. Cereb. Cort. *18*, 2416–2438.
10. Hershberger, W. (1970). Attached-shadow orientation perceived as depth by chickens reared in an environment illuminated from below. J. Comp. Physiol. Psychol. *73*, 407–411.

Evolution: A Plant Plastid Genome that Has Forsaken Guanine and Cytosine

David Roy Smith
Department of Biology, University of Western Ontario, London, ON, Canada
Correspondence: dsmit242@uwo.ca
https://doi.org/10.1016/j.cub.2018.12.025

The plastid genomes of the non-photosynthetic plants *Balanophora reflexa* and *B. laxiflora* are among the most GC-biased genomes observed to date. A new study shows that ~80% of the plastid-derived proteome is represented by only six amino acids, and several genes are in excess of 95% AT.

> *"The road of excess leads to the palace of wisdom… You never know what is enough until you know what is more than enough."*
>
> –William Blake

As a long-time connoisseur and surveyor of unconventional organelle genomes, I thought I had seen it all. However, the recent sequencing and characterization of two plastid DNAs (ptDNAs) from the holoparasitic plant genus *Balanophora* (Figure 1) has proved me wrong and raised the bar of what defines an extreme genome [1]. With AT compositions of 88.4% and 87.8%, the *B. reflexa* and *B. laxiflora* plastomes have a smaller proportion of GC base pairs than any other ptDNA explored to date. Even more remarkable, the AT bias is most prominent in the true heart and soul of these genomes: the protein-coding genes.

It stands to reason that coding DNA should contain at least some guanine and

Figure 1. *Balanophora laxiflora*.
Image of the non-photosynthetic, holoparasitic plant *Balanophora laxiflora*, which has one of the most AT-rich plastid genomes on record. Photo credit: Huei-Jiun Su.

cytosine in order to encode the correct cohort of amino acids needed for making functional proteins. I guess the *Balanophora* ptDNAs did not get this memo. Darwin help us, the *ycf2* gene from both species is 98% AT! Or, put differently: across the ~750 nt that make up this plastid gene, there are fewer than eighteen sites containing a G or C. Several other plastid genes are also ≥95% AT, including *ycf1*, *rps2*, and *rps18*, and the overall AT content of the protein-coding regions is an astonishing 91%, putting them in the running for the most AT-rich gene sets of any sequenced genome.

The nucleotide composition statistics get even more startling when focusing in on the third position of synonymous codons — sites that tend to be under less selective constraints than their first and second position counterparts. Weighing in at a measly 1% GC, synonymous sites in the *Balanophora* plastid genomes have one-tenth the number of Gs and Cs as those from the next most GC-poor angiosperm ptDNA. Consequently, the proteins encoded by these plastids are highly biased in amino acids represented by AT-rich codons. In *B. laxiflora*, for example, 80% of the plastid-derived proteome is represented by only six amino acids (Asn, Ile, Leu, Lys, Phe, and Tyr), all of which can be encoded by codons lacking G or C. Another peculiar outcome of such skewed AT contents is that the nucleotide identity between *B. reflexa* and *B. laxiflora* is greater than the amino-acid identity for every one of the plastid protein genes, thus breaking one of the standard rules of molecular evolution.

At this point, you must be saying to yourself: "Surely, the *Balanophora* plastid protein-coding regions cannot get any stranger". Think again. *B. reflexa* and *B. laxiflora* have acquired a never-before-seen change to the genetic code whereby the canonical stop codon UGA signifies tryptophan. Not only is this the first documented instance of such a change, but it is one of the few clear-cut examples of a nonstandard genetic code in a plastid [2–4]. To top it off, the tRNA that now recognizes this code change is not located in the plastome. In fact, not a single tRNA is encoded in the *Balanophora* ptDNA, another first for a plastid genome.

With no tRNAs and only 15 protein genes, it is no surprise that the circular-mapping *Balanophora* plastomes are very small, measuring just 15.5 kb. But this number, despite being the fourth smallest ever recorded for ptDNA [5,6], does not do justice to the extreme level of compaction occurring in these genomes. About a third of the protein genes in *B. reflexa* and *B. laxiflora* overlap with one another by 4–15 nt, and two thirds are significantly shrunken relative to their counterparts in other angiosperms. For instance, *ycf2* is 6000 nt (88%) shorter — yes, you read that right — than its homolog in *Schoepfia jasmiodora*, a close photosynthetic relative of *Balanophora*.

Given the genetic upheaval of the *Balanophora* protein-coding regions, it would be fair to ask: are they even functional? The answer appears to be yes. All the genes are transcribed and, based on a pairwise comparison between *B. reflexa* and *B. laxiflora*, they have continued to evolve under purifying selection, which is a telltale sign that they have a purpose. Moreover, staining revealed numerous oil bodies in the *Balanophora* plastids, suggesting that these non-photosynthetic organelles are actively synthesizing fatty acids.

If all of this leaves you scratching your head, you are not alone. The authors of the *Balanophora* plastome study considered various hypotheses for the prodigious proportions of A and T in these genomes. First, they convincingly ruled out two of the most common explanations for extremely biased nucleotide compositions: selection for translationally optimal codons and selection driven by limited nitrogen availability and/or energetic costs. Indeed, neither the *B. reflexa* nor *B. laxiflora* plastid proteins are depleted in nitrogen-rich or energetically expensive amino acids, and some of their most frequently employed codons are likely translated via superwobbling, which is relatively inefficient [7]. Ultimately, the authors favored the idea that persistent and long-term genome-wide AT mutation pressure gradually erased and replaced most of the guanine and cytosine sites from the *Balanophora* ptDNAs. Such a non-adaptive hypothesis might leave some readers wanting more, but one should never underestimate the power of drift and mutation in shaping genomic architectures, especially those of organelles [8].

Mutational pressure can also help explain the novel genetic code in the *Balanophora* plastids. Under the codon-capture hypothesis [9], the first step in reassigning UAG to tryptophan is the removal of all UAG stop codons from the plastome via mutation to UAA. This is followed by duplication of the plastid $tRNA^{Trp}$ gene and mutation to one of the copies so that UAG (in addition to UGG) is deciphered as tryptophan. Eventually, all UGG codons are replaced by UAG and, voilà, the new code is locked in place. With the exception of gene duplication, an AT-biased mutational process would assist rather than obstruct all of these steps. And, if you haven't already guessed, not a single UGG codon was found in the *B. reflexa* and *B. laxiflora* plastomes. It is noteworthy that the nuclear genes in *Balanophora* are not particularly enriched in AT, show only a small codon-usage bias, and still use UGG for Trp and UAG as a stop codon. The same also appears to be true of the mitochondrial genome (based on the analysis of a few

genes), which is surprising because mitochondrial and plastid genomes often get pulled in similar evolutionary directions, particularly those from the same species [8].

Any plastome that is housed in a non-photosynthetic species faces the threat of outright loss.

The holoparasitic angiosperm *Rafflesia lagascae* is thought to have abandoned its ptDNA [10] as has the colourless green alga *Polytomella* [11]. Arguably, a key prerequisite for relinquishing a plastome (while still maintaining a plastid) is the genetic transfer of functionally crucial ptDNA-encoded genes to the nuclear genome and the successful targeting of their protein products back to the plastid [12]. Such an outcome seems unlikely for *Balanophora*: presumably, the massive AT-bias and novel genetic code of the ptDNA would prevent successful transfer of any plastid-encoded gene to the nucleus. In other words, the amazing plastomes of *Balanophora* are here to stay. Whether or not they have reached peak AT content or are inching their way back to a more moderate nucleotide composition only evolutionary time will tell.

REFERENCES

1. Su, H.J., Barkman, T.J., Hao, W., Jones, S.S., Naumann, J., Skippington, E., Wafula, E.K., Hu, J.-M., Palmer, J.D., and dePamphilis, C.W. (2019). Novel genetic code and record-setting AT-richness in the highly reduced plastid genome of the holoparasitic plant Balanophora. Proc. Natl. Acad. Sci. USA *116*, 934–943.

2. Lang-Unnasch, N., and Aiello, D.P. (1999). Sequence evidence for an altered genetic code in the *Neospora caninum* plastid. Int. J. Parasitol. *29*, 1557–1562.

3. Tang, K., Guo, Y., Zhang, L., Rowe, L.A., Roellig, D.M., Frace, M.A., Li, N., Liu, S., Feng, Y., and Xiao, L. (2015). Genetic similarities between *Cyclospora cayetanensis* and cecum-infecting avian *Eimeria* spp. in apicoplast and mitochondrial genomes. Parasit. Vectors *8*, 358.

4. Moore, R.B., Oborník, M., Janouškovec, J., Chrudimský, T., Vancová, M., Green, D.H., Wright, S.W., Davies, N.W., Bolch, C.J., Heimann, K., and Šlapeta, J. (2008). A photosynthetic alveolate closely related to apicomplexan parasites. Nature *451*, 959–963.

5. Bellot, S., and Renner, S.S. (2015). The plastomes of two species in the endoparasite genus *Pilostyles* (Apodanthaceae) each retain just five or six possibly functional genes. Genome Biol. Evol. *8*, 189–201.

6. Petersen, G., Zervas, A., Pedersen, H.A.E., and Seberg, O. (2018). Genome reports: contracted genes and dwarfed plastome in mycoheterotrophic *Sciaphila thaidanica* (Triuridaceae, Pandanales). Genome Biol. Evol. *10*, 976–981.

7. Alkatib, S., Scharff, L.B., Rogalski, M., Fleischmann, T.T., Matthes, A., Seeger, S., Schöttler, M.A., Ruf, S., and Bock, R. (2012). The contributions of wobbling and superwobbling to the reading of the genetic code. PLoS Genet. *8*, e1003076.

8. Smith, D.R., and Keeling, P.J. (2015). Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. Proc. Natl. Acad. Sci. USA *112*, 10177–10184.

9. Osawa, S., and Jukes, T.H. (1999). Codon reassignment (codon capture) in evolution. J. Mol. Evol. *28*, 271–278.

10. Molina, J., Hazzouri, K.M., Nickrent, D., Geisler, M., Meyer, R.S., Pentony, M.M., Flowers, J.M., Pelser, P., Barcelona, J., Inovejas, S.A., and Uy, I. (2014). Possible loss of the chloroplast genome in the parasitic flowering plant *Rafflesia lagascae* (Rafflesiaceae). Mol. Biol. Evol. *31*, 793–803.

11. Smith, D.R., and Lee, R.W. (2014). A plastid without a genome: evidence from the nonphotosynthetic green algal genus *Polytomella*. Plant Physiol. *164*, 1812–1819.

12. Janouškovec, J., Tikhonenkov, D.V., Burki, F., Howe, A.T., Kolísko, M., Mylnikov, A.P., and Keeling, P.J. (2015). Factors mediating plastid dependency and the origins of parasitism in apicomplexans and their close relatives. Proc. Natl. Acad. Sci. USA *112*, 10200–10207.

Developmental Biology: Go with the Flow to Keep the Body Straight

Daniel T. Grimes
Institute of Molecular Biology, Department of Biology, University of Oregon, Eugene, OR 97403, USA
Correspondence: dtgrimes@uoregon.edu
https://doi.org/10.1016/j.cub.2018.12.011

It has long been noticed that zebrafish defective in ciliary beating develop abnormal body curvatures. Recently, insights into how cilia keep the body straight have emerged, with implications for understanding human scoliosis.

A straight head-to-tail animal body axis allows for coordinated, directional movement. But how the body first attains and then maintains straightness during development, growth and adulthood is little understood. The need to comprehend how the body stays straight is underscored by the remarkable prevalence of scoliotic spinal curves in human populations. With this goal, researchers have turned to the zebrafish model organism. Three recent studies [1–3] on zebrafish provide fresh mechanistic understanding of body straightening and provide a deeper appreciation of the causes of scoliosis.

The story starts with a simple but striking phenotype: curly tail down. For many years, researchers studying mutant zebrafish embryos had noticed this common defect, where the body axis is not straightened and the tail instead loops downwards in a ventral curl. Several curly tail down mutant lines were recovered