killer whale is a member of an ecotype, and the ecotype's diet may be much more restricted, witness the focus on Chinook salmon of the southern residents [17]. The ecotypes differ in other ways. For instance, members of the North Pacific 'transient' ecotype who can be seen in the same waters as the southern residents, eat marine mammals rather than salmon, have larger ranges and are much less vocal [17]. The differences are so substantial that geneticists have suggested that the different killer whale ecotypes should be considered species or subspecies [18].

The benefits of older mothers for survival and leadership [8,12] only refer to the 'southern' community of the resident, salmon-eating ecotype. Are they also present in other communities of resident ecotype killer whales, which also eat salmon? The residents have a very unusual social system in which neither sex leaves its mother's group: "Momma's boys and girls" [19]. This arrangement leads to the increasing presence of kin as females age, and strong theoretical support for menopause [9]. The mammal-eating transient killer whales are less rigid about spending their whole lives with mother [20], as may be other ecotypes whose social systems are even less studied. Do they have menopause? And who leads? Comparative studies among killer whale ecotypes have much to tell us.

Even more broadly, there are about 87 species of cetaceans — whales and dolphins — with a great variety of diets, habitats and social systems. The short-finned pilot whale, another matrilineal species, seems to have a menopause as pronounced as that in humans and resident killer whales. In other species, such as sperm whales, there are strong indications that reproduction ceases for older females, while many species, including the porpoises and baleen whales, do not have menopause [5]. The social systems of only four of these species of Cetacea have been studied in much detail [2]. There is so much to learn.

We now have a remarkable insight into the lives of the resident killer whales, capped by the study reported in this issue [8], showing how much can be learned by long-term, persistent observation. New technology is adding genomics, as well as fine scale behaviour and physiology from suction-cup tags. The evolution of the southern resident population should before long be traceable at the level of individuals, as they react to their dynamic physical, biotic and social environments.

References

1. Williams, G.C. (1957). Pleiotropy, natural selection, and the evolution of senescence. Evolution *11*, 398–411.
2. Whitehead, H., and Rendell, L. (2015). The cultural lives of whales and dolphins (Chicago, IL: University of Chicago Press).
3. Austad, S.N. (1994). Menopause: an evolutionary perspective. Exp. Gerontol. *29*, 255–263.
4. Hawkes, K., O'Connell, J.F., Blurton Jones, N.G., Alvarez, H., and Charnov, E.L. (1998). Grandmothering, menopause, and the evolution of human life histories. Proc. Natl. Acad. Sci. USA *95*, 1336–1339.
5. Marsh, H., and Kasuya, T. (1986). Evidence for reproductive senescence in female cetaceans. Rpt. Int. Whal. Commn (Spec. Issue) *8*, 57–74.
6. Ford, J.K.B., Ellis, G.M., and Balcomb, K.C. (2000). Killer whales (Vancouver, British Columbia: UBC Press).
7. Hawkes, K. (2004). Human longevity: the grandmother effect. Nature *428*, 128–129.
8. Brent, L.J.N., Franks, D.W., Foster, E.A., Balcomb, K.C., Cant, M.A., and Croft, D.P. (2015). Ecological knowledge, leadership, and the evolution of menopause in killer whales. Curr. Biol. *25*, 746–750.
9. Johnstone, R.A., and Cant, M.A. (2010). The evolution of menopause in cetaceans and humans: the role of demography. Proc. R. Soc. Lond. B *277*, 3765–3771.
10. Lahdenperä, M., Lummaa, V., Helle, S., Tremblay, M., and Russell, A.F. (2004). Fitness benefits of prolonged post-reproductive lifespan in women. Nature *428*, 178–181.
11. Lahdenperä, M., Russell, A.F., Tremblay, M., and Lummaa, V. (2011). Selection on menopause in two premodern human populations: no evidence for the mother hypothesis. Evolution *65*, 476–489.
12. Foster, E.A., Franks, D.W., Mazzi, S., Darden, S.K., Balcomb, K.C., Ford, J.K.B., and Croft, D.P. (2012). Adaptive prolonged postreproductive life span in killer whales. Science *337*, 1313.
13. Diamond, J. (2001). Unwritten knowledge. Nature *410*, 521.
14. McAuliffe, K., and Whitehead, H. (2005). Eusociality, menopause and information in matrilineal whales. Tr. Ecol. Evol. *20*, 650.
15. Ford, J.K., Ellis, G.M., Olesiuk, P.F., and Balcomb, K.C. (2010). Linking killer whale survival and prey abundance: food limitation in the oceans' apex predator? Biol. Lett. *6*, 139–142.
16. McComb, K., Shannon, G., Durant, S.M., Sayialel, K., Slotow, R., Poole, J., and Moss, C. (2011). Leadership in elephants: the adaptive value of age. Proc. R. Soc. Lond. B *278*, 3270–3276.
17. Riesch, R., Barrett-Lennard, L.G., Ellis, G.M., Ford, J.K.B., and Deecke, V.B. (2012). Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? Biol. J. Linn. Soc. *106*, 1–17.
18. Morin, P.A., Archer, F.I., Foote, A.D., Vilstrup, J., Allen, E.E., Wade, P., Durban, J., Parsons, K., Pitman, R., and Li, L. (2010). Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Res. *20*, 908–916.
19. Bigg, M.A., Olesiuk, P.F., Ellis, G.M., Ford, J.K.B., and Balcomb, K.C. (1990). Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Rpt. Int. Whal. Commn (Spec. Issue) *12*, 383–405.
20. Baird, R.W., and Whitehead, H. (2000). Social organization of mammal-eating killer whales: group stability and dispersal patterns. Can. J. Zool. *78*, 2096–2105.

Biology Department, Dalhousie University, 1355 Oxford St, Halifax, Nova Scotia, Canada B3H4J1.
E-mail: hwhitehe@dal.ca

http://dx.doi.org/10.1016/j.cub.2015.02.002

Microbial Diversity: A Bonanza of Phyla

Metagenomics and single-cell genomics are now the gold standard for exploring microbial diversity. A new study focusing on enigmatic ultra-small archaea greatly expands known genetic diversity within Archaea, and reports the first complete archaeal genomes reconstructed from metagenomic data only.

Laura Eme and W. Ford Doolittle*

The ribosomal RNA (rRNA)-based universal Tree of Life that appears in biology textbooks, the signal achievement of Carl Woese and his school [1], separates the living world into three domains — Bacteria, Archaea and Eukarya. Characteristically this tree shows long unbranched 'trunks' leading to each of the three domains, these trunks representing the gaps between them (Figure 1A). The gaps were initially interpreted by Woese to indicate a different tempo or mode of evolution early on, before the formation of the domains. But two things are inevitable about trees: first, that if speciation and extinction are in balance, considerable

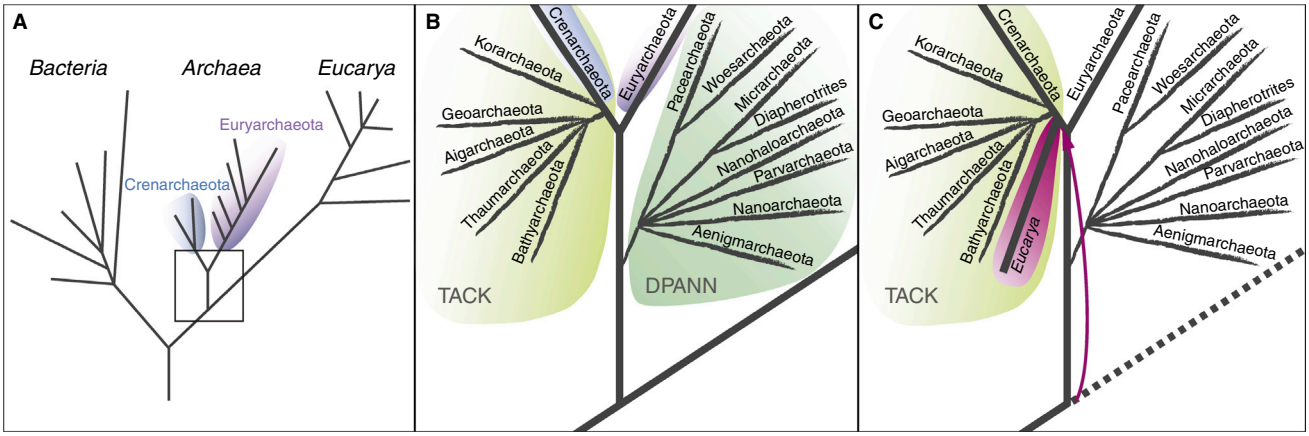

Figure 1. The evolving Tree of Life.

(A) The three domain Tree of Life, as conceived by Woese and colleagues [1]. Area in square is expanded in panel B. (B) Placement of more recently designated archaeal phyla and superphyla, as by Castelle *et al.* [3]. (C) Traditional placement of the branch leading to eukaryotes is shown as a dotted line. Recent work indicates that eukaryotes may instead emerge from within or at the base of the archaeal TACK superphylum [13].

gaps separating primary divisions are not unlikely [2], and second that further discoveries can only fill in, not extend, these gaps. In a new study in this issue of *Current Biology*, Castelle *et al.* [3] tentatively fill some gaps at the base of the domain Archaea (Figure 1B).

Inexorably Advancing Methodology

As long as further discoveries were limited by the need to culture organisms and extract their ribosomal RNAs (rRNAs) or the genes encoding them, gap filling was slow and biased towards easily cultivated groups of interest for other reasons (disease causation, for instance). The sequencing, after PCR amplification, of rRNA genes present in unfractionated DNA made straight from environmental samples ('phylotyping' of oceans, soils, sewers and shower curtains) broke us free of that constraint, and coincidentally revolutionized environmental microbiology [4]. More than 80% of the more than three million 16S rRNA sequences currently available are derived from 'environmental PCR'. When such sequences fail to cluster with known 'phyla' having cultured members (ideally with sequenced genomes), they are often taken to represent new 'candidate phyla', which collectively and (perhaps regrettably) have been called 'microbial dark matter', or MDM. An estimate of the number of prokaryotic phyla that may actually be 'out there' (light and dark) exceeds 130 [5].

Yarza *et al.* [5] base this estimate on phyla so far named, which on average show a within-phylum 16S sequence similarity of 84%. There seems not to be a solid basis for the designation of phylum status for prokaryotes other than such arbitrary thresholds for marker sequence similarity. With animal phyla, there is in principle a shared 'body plan' as well as monophyly, and Gribaldo and Brochier-Armanet [6] suggested that we need such a phenotypic characterization for prokaryotes.

Two newer methods are the next steps in the ongoing taxonomic revolution, and can be used specifically to address the MDM, which by definition lacks cultured representatives. Moreover, they allow greater phylogenetic resolution (using concatenated sequences of core protein-coding genes shared by many taxa) and some approach to what might be phylum-defining phenotypes. The first method — metagenomics — looks at sequencing reads of random fragments of DNA from all genomes present, albeit at different abundances, in a sample taken straight from an environment. Metagenomes can be assembled into individual genomes more readily if closely related complete genome sequences are available for ordering contigs. But even without that, binning methods based on nucleotide composition and other sequence characteristics, or read depth within and between samples [7], permit assembly of some species genomes from the MDM. If there is considerable within-species diversity in the sample, one gets 'pangenomes'.

The second method produces SAGs (single amplified genomes), by DNA amplification following isolation of single cells with methods such as fluorescence-activated cell sorting. PCR of phylogenetic markers like 16S from SAGs allows taxonomic identification, and detection of presumed single copy core genes assesses completeness. Metagenomics and SAG work even better together, SAGs allowing genomic assignment of unlinked metagenomic contigs, and the truer representation of sequence abundance in metagenomic data correcting inevitable biases in extensive genome amplification in SAGs [8].

Filling in Archaea

Initially and for some time, Archaea was thought to comprise two phyla (or perhaps kingdoms), the Crenarchaeota and the Euryarchaeota, both consisting of 'extremophiles' (*e.g.*, hyperthermophiles, halophiles, and methanogens). The advances of 16S rRNA 'phylotyping' and metagenomics have since shown that archaea are in fact extremely abundant in moderate environments including soils, sediments, oceans, and freshwater. Concomitantly, these environmental surveys revealed new

major lineages, some representing putative new phyla, such as Korarchaeota, Thaumarchaeota, and 'Aigarchaeota'. Collectively, these are referred to as the TACK group of phyla [9].

Metagenomics and single-cell genomics have also revealed several enigmatic lineages of uncultured tiny archaea from diverse environments. These organisms display very small cells (~400–500 nm) and genomes (~550 genes for *Nanoarcheum equitans*, ~1000 for *Candidatus* '*Parvarchaeum acidophilus*' and '*Micrarchaeum acidiphilum*', as well as fast-evolving gene sequences. A deep-branching phylogenetic position has been proposed for many of them [10], and Rinke *et al.* [11] have even argued for the existence of a superphylum ('DPANN') containing all ultrasmall archaea (*i.e.*, Diapherotrites, *Ca*. '*Parvarchaeum acidophilus*' and '*Micrarchaeum acidiphilum*', Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota) — the root of the tree falling between this superphylum and all other members of Archaea. However, others argue for alternative placements of some of these lineages (*e.g.*, [9,12]), their apparent deep-branching position being potentially artefactually caused by their peculiar and small gene content combined with the fast rate of evolution of these genes.

A recent megasurvey using SAG technology [11] and calling itself GEBA-MDM looks at 200 SAGs from 20 bacterial and archaeal phyla, venturing to cluster some of these as phyla or superphyla, some with unifying (if minimal) characteristic physiologies, as inferred from genomic composition. Castelle *et al.*, on the other hand, rely exclusively on genomic sampling of unisolated archaea through metagenomic methods. They specifically target nanosized archaea (0.1–1.2 micron) from aquifer sediments and associated anoxic groundwater [3]. Phylogenetic analyses based on 15 ribosomal proteins and 153 newly identified taxa reveal that, in addition to newly uncovered TACK and euryarchaeotal organisms, most of these novel archaea fall within two highly supported and previously undescribed monophyletic clades. Based on their genetic distance to all other sequenced archaea, as well as the wide genetic diversity within them (up to 20% 16S rRNA divergence), the authors propose to consider them phyla, tentatively named 'Woesearchaeota' and 'Pacearchaeota'. They also suggest a deep-branching position for these two novel phyla and their affiliation to the DPANN superphylum, the root of the Archaea tree falling within the latter.

In addition, Castelle and colleagues use clustering methods based on tetranucleotide sequence composition to reconstruct 14 draft and 2 complete genomes of nanosized archaea, the first closed archaeal genomes obtained from metagenomic data alone. In particular, these include the first complete Diapherotrites and Woesearcheota genomes, as well as several advanced drafts for Pacearchaeota and Aenigmarchaeota, drastically expanding the known genomic diversity of Archaea. The authors provide detailed metabolic analyses of these reduced genomes, which point to a primary contribution to carbon and hydrogen biogeochemical cycles, likely associated with symbiotic and/or fermentation-based lifestyles.

Without doubt, this impressive amount of new sequence data will fuel the debate on the monophyly of DPANN, and on the position of nanosized organisms relative to the root of the archaeal tree. The authors tentatively provide an answer that will have to be confirmed by extensive analyses in order to investigate potential phylogenetic artefacts caused by the peculiarity of these genomes. Indeed, the placement of these organisms is of crucial importance when it comes to inferring the entire set of characteristics of the Last archaeal common ancestor (LACA), and retracing the evolution of extant archaeal lineages since the time of LACA. If the root is confirmed to lie within early-branching nanosized archaea, we will have to entertain the hypothesis of a LACA with a small genome and a subsequent rapid gene gain in the branch leading to the ancestor of other archaeal groups (*i.e.*, TACK and Euryarchaeota), which possess wider gene repertoires. Conversely, if further analyses challenge the deep position of the "DPANN" Archaea (and thus the notion that the root of the archaeal tree lies within them), this will imply that they evolved from more complex, gene-rich ancestors by genome reduction. Significantly, resolving the phylogenetic position of these organisms and investigating their key cellular features is also central to the question of the origin of the eukaryotic lineage (Figure 1C).

Until a few years ago, the study of archaeal evolution was based on a mere hundred genomes from cultivable organisms. The ability to reconstruct complete genomes from environmental samples using metagenomic data and single cell genomics promises a drastic acceleration in understanding the diversity and complex evolutionary history of this 'third domain of Life', and its links to Bacteria and Eukarya.

References

1. Woese, C.R., Kandler, O., and Wheelis, M.L. (1990). Toward a natural system of organisms: Proposal for the domains Archaea. Bacteria and Eukarya. Proc. Natl. Acad. Sci. USA *87*, 4576–4579.
2. Zhaxybayeva, O., and Gogarten, J.P. (2004). Cladogenesis, coalescence and the evolution of the three domains of life. Trends Genet. *20*, 182–187.
3. Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., Frischkorn, K.R., Tringe, S.G., Singh, A., Markillie, L.M., *et al*. (2014). Genomic expansion of domain Archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. Curr. Biol. *25*, 690–701.
4. Olsen, G.J., Lane, D.J., Giovannoni, S.J., Pace, N.R., and Stahl, D.A. (1986). Microbial RNA and ecology: a ribosomal RNA approach. Annu. Rev. Microbiol. *40*, 337–365.
5. Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzéby, J., Amann, R., and Rosselló-Móra, R. (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat. Rev. Microbiol. *12*, 635–645.
6. Gribaldo, S., and Brochier-Armanet, C. (2012). Time for order in microbial systematics. Trends Microbiol. *20*, 209–210.
7. Albertsen, M., Hugenholtz, P., Skarshewski, A., Nielsen, K.L., Tyson, G.W., and Nielsen, P.H. (2014). Genome sequences of rare, uncultured, bacteria obtained by differential coverage binning of multiple metagenomes. Nature Biotechnol. *11*, 533–538.
8. Hedlund, B.P., Dodsworth, J.A., Murugapiran, S.K., Rinke, C., and Woyke, T. (2014). Impact of single-cell genomics and metagenomics on the emerging view of extremophile "microbial dark matter". Extremophiles *18*, 865–875.
9. Brochier-Armanet, C., Forterre, P., and Gribaldo, S. (2011). Phylogeny and evolution of the Archaea: one hundred genomes later. Curr. Opin. Microbiol. *14*, 274–281.
10. Baker, B.J., Comolli, L.R., Dick, G.J., Hauser, L.J., Hyatt, D., Dill, B.D., Landc, M.L., VerBerkmoesd, N.C., Hettich, R.L., and Banfield, J.F. (2010). Enigmatic, ultrasmall, uncultivated Archaea. Proc. Nat. Acad. Sci. USA *107*, 8806–8811.

11. Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.F., Darling, A., Malfatti, S., Swan, B.K., Gies, E.A., Dodsworth, J.A., Hedlund, B.P., Tsiamis, G., Sievert, S.M., Liu, W.T., Eisen, J.A., Hallam, S.J., Kyrpides, N.C., Stepanauskas, R., Rubin, E.M., Hugenholtz, P., and Woyke, T. (2013). Insights into the phylogeny and coding potential of microbial dark matter. Nature *499*, 431–437.
12. Narasingarao, P., Podell, S., Ugalde, J.A., Brochier-Armanet, C., Emerson, J.B., Brocks, J.J., Heidelberg, K.B., Banfield, J.F., and Allen, E.E. (2011). De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. ISME J. *6*, 81–93.
13. Williams, T.A., and Embley, T.M. (2014). Archaeal "dark matter" and the origin of eukaryotes. Genome Biol. Evol. *6*, 474–481.

Department of Biochemistry and Molecular Biology, Dalhousie University, P.O. Box 15000, Halifax, Nova Scotia B3H 4R2, Canada.
*E-mail: ford@dal.ca

http://dx.doi.org/10.1016/j.cub.2014.12.044

Growth Control: Re-examining Zyxin's Role in the Hippo Pathway

The Hippo pathway is a conserved regulator of organ growth that computes information from the cellular microenvironment. A new study examines the role of the Hippo pathway protein Zyxin and finds that it antagonises Expanded to modulate F-actin and organ size.

Kieran F. Harvey

Cellular signalling pathways commonly transmit information from the extracellular environment to the nucleus to modulate transcription and elicit a biological response. The Hippo pathway is one of the most recently identified and intensely studied signalling pathways. Unlike most pathways, which transmit information downstream of diffusible extracellular ligands that bind to transmembrane receptor proteins, the Hippo pathway appears predominantly to convey information about the 'cellular neighbourhood' of tissues [1–3]. Hippo signalling has been shown to be influenced by G-protein-coupled receptors [4], which are regulated by diffusible ligands, but is also controlled by transmembrane proteins that form ligand–receptor pairs between neighbouring cells (e.g. the Fat and Dachsous cadherins, Crumbs and Echinoid) [5]. The Hippo pathway also responds to key cell biological properties, such as cell polarity and cell adhesion [1–3]. In addition, this pathway is sensitive to mechanical properties of cells and tissues, and it has been touted as a key integrator of tissue mechanics, in the context of both organ size control and tumorigenesis [6]. In this role the Hippo pathway is thought to be controlled by the tensile state of the actin cytoskeleton [2,6].

Two of the best-studied upstream regulators of the Hippo pathway are Fat and Expanded. Fat engages in bidirectional signalling with its ligand Dachsous and signals via several proteins, including the atypical myosin Dachs and the casein kinase Discs overgrown [7,8]. Expanded forms complexes with multiple Hippo pathway proteins, including the upstream regulators Merlin and Kibra and the core pathway members Warts, Hippo, Salvador and Yorkie [9–11]. Expanded can repress Yorkie by direct binding and also by activating the kinase Warts, which phosphorylates and inhibits Yorkie [9–11]. Despite rapid advances in the past decade or so, many aspects of Hippo signalling are still shrouded in uncertainty.

In a study published in this issue of *Current Biology*, Gaspar *et al.* [12] address the role of Zyxin, a protein that has been independently linked to both Hippo signalling and mechanotransduction, and suggest that Zyxin might present a nexus between the two. Zyxin possesses a triple LIM domain that has been shown to mediate its association with focal adhesions and actin fibres. It also binds to the F-actin polymerisation factors Enabled and VASP in both *Drosophila melanogaster* and mammalian cultured cells [13,14]. Zyxin is recruited to F-actin that has been compromised by mechanical force and is proposed to induce actin fibre repair at least in part by recruiting Enabled/VASP [15]. Zyxin also controls tissue growth via the Hippo pathway [16]. Based largely on RNA interference studies, Zyxin had been proposed to function downstream of the Fat branch of the Hippo pathway, but independently of Expanded [16]. In particular, biochemical experiments showed that Zyxin bound to both Dachs and the key kinase Warts and that Zyxin functions with Dachs to limit Warts levels via an as yet unknown mechanism [16].

The genomic localisation of *D. melanogaster zyxin* (on the relatively small and less genetically tractable fourth chromosome) has hindered the ability to study this gene using traditional loss-of-function alleles. Gaspar *et al.* [12] have now overcome this challenge by using genome editing to generate null *zyxin* mutant flies, which allowed them to reappraise its role in Hippo signalling and tissue growth. Based on several phenotypes of *zyxin* null tissue, and the fact that *zyxin* overexpression strongly rescued phenotypes caused by *expanded* overexpression, they conclude that Zyxin has a major role in antagonising Expanded. Key data supporting their claims include the demonstration that *zyxin* loss strongly suppressed imaginal disc overgrowth and defects in eye differentiation associated with *expanded* loss but not *fat* loss, while *zyxin* overexpression more robustly counteracted growth retardation induced by *expanded* than that induced by *fat.* Perhaps most compelling is the finding that *zyxin* loss brought *expanded*, but not *fat*, mutant flies back to life [12].

It is difficult to fully reconcile the seemingly different results presented in this study and that from Rauskolb *et al.* [16], but it should be recognised that, whilst RNA interference has revolutionised the study of gene function in many organisms, including *D. melanogaster*, it has many