Pogo-like Transposases Have Been Repeatedly Domesticated into CENP-B-Related Proteins

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Abstract

The centromere is a chromatin region that is required for accurate inheritance of eukaryotic chromosomes during cell divisions. Among the different centromere-associated proteins (CENP) identified, CENP-B has been independently domesticated from a pogo-like transposase twice: Once in mammals and once in fission yeast. Recently, a third independent domestication restricted to holocentric lepidoptera has been described. In this work, we take advantage of the high-quality genome sequence and the wealth of functional information available for Drosophila melanogaster to further investigate the possibility of additional independent domestications of pogo-like transposases into host CENP-B related proteins. Our results showed that CENP-B related genes are not restricted to holocentric insects. Furthermore, we showed that at least three independent domestications of pogo-like transposases have occurred in metazoans. Our results highlight the importance of transposable elements as raw material for the recurrent evolution of important cellular functions.

Key words: pogo, Drosophila, exaptation, functional domain, holocentric chromosomes.

Centromere-Associated Protein B Homologs Are Present in Mammals, Fission Yeast, and Holocentric Lepidoptera

CENP-B is one of the earliest described cases of transposable element (TE) exaptations in the human genome (Tudor et al. 1992; Smit 1996). Human CENP-B has extensive sequence and domain similarity to transposases encoded by the pogo superfamily of TEs. It is widespread and highly conserved in mammals, whereas it is undetectable in other metazoans (Casola et al. 2008). Other than in mammals, three CENP-B homologs have been described in fission yeast: Abp1 (Autonomous replicating sequence-binding protein 1), Cbh1 (CENP-B homolog 1), and Cbh2 (CENP-B homolog 2). Fission yeast and human CENP-B proteins are functionally related. Fission yeast CENP-B homologs show partially redundant function in the formation of centromeric heterochromatin and in chromosome segregation (Irelan et al. 2001). They also play a role in the silencing of TEs and TE-associated genes (Cam et al. 2008; Lorenz et al. 2012) and in DNA replication (Zaratiegui et al. 2011). In humans, although the role of CENP-B has been controversial (Marshall and Choo 2012), it has been recently shown that CENP-B provides an alternative redundant pathway for kinetochore formation in vivo (Fachinetti et al. 2013). Sequence and functional relationship between mammal and fission yeast CENP-B homologs is the result of convergent domestication: Different pogo-like transposases have been exapted independently in the two lineages to give rise to host proteins with centromere-binding activity (Casola et al. 2008).

Recently, a CENP-B homolog has been described in the holocentric lepidoptera Spodoptera frugiperda (d’Alencón et al. 2011). Although in most eukaryotes the kinetochore protein complex, connecting chromosomes to spindle microtubules during cell division, usually binds to a single locus called the centromere, in holocentric chromosomes kinetochore proteins bind along the entire length of the chromosomes. Spodoptera frugiperda CENP-B ability to bind in vivo to a retrotransposon derived sequence and its nuclear localization suggest that this protein is functionally related to other CENP-B homologs (d’Alencón et al. 2011). Orthologs of S. frugiperda CENP-B have been identified in other holocentric lepidoptera, Bombyx mori and Helicoverpa armigera, but not in other invertebrates. These findings suggest that there has been a third convergent domestication of a transposase into a CENP-B-related (CR) protein that appears to be restricted to holocentric lepidopteran species (d’Alencón et al. 2011).
These results prompted us to further investigate whether CR proteins can be identified in the Drosophila melanogaster genome. Drosophila melanogaster has one of the highest quality genomes in terms of sequence and functional annotation (St Pierre et al. 2013), and its DNA is organized in nonholocentric chromosomes: Two metacentric and two telocentric ones.

**CAG Is the Closest CR Protein in D. melanogaster**

To identify CR proteins in D. melanogaster, we used the protein sequences of the previously identified CENP-B homologs and D. melanogaster pogo transposase as queries in BLASTp searches against the D. melanogaster protein database. As expected, we found that the pogo transposase was the best hit in all searches (20–30% identity, e values 4e$^{-34}$–3e$^{-12}$). We also found that CAG (CG12346) was the only host protein showing local significant sequence similarity with human CENP-B (34% identity, e value 4e$^{-17}$), fission yeast CbH1 (26% identity, e value 2e$^{-05}$), and Drosophila pogo transposase (26% identity, e value 9e$^{-10}$). Reciprocal BLAST searches using CAG as a query confirmed that the closest sequence in fission yeast is CbH1 (25% identity, e value 7e$^{-06}$). On the other hand, CAG shows significant sequence homology with 40 blast hits in human, being the host genes TIGD6 (34% identity, e value 3e$^{-18}$) and CENP-B (34% identity, e value 5e$^{-18}$) the highest scoring hits. Although four other proteins containing CENP-B domains have been described in D. melanogaster, we could not detect them in an exhaustive search using BLASTp, tBLASTN, and HMMER, suggesting that they are not closely related to CAG and previously described CR proteins (Benchabane et al. 2011).

To further determine that CAG is a transposon-derived gene and not a transposon remnant, we followed the conservative approach proposed by Feschotte and Pritham (2007). CAG fulfills the six criteria proposed by these authors. First, we did not find evidence of transposon hallmarks, that is, Terminal Inverted Repeats (TIRs), in CAG flanking regions, suggesting that CAG is not a transposon. Second, CAG shows significant sequence and domain architecture similarities (see below) with pogo transposase and other transposase-derived genes suggesting that it has a transposon origin. Third, the coding capacity of CAG is intact and it is evolving under functional constrain contrary to TE-coding regions of nonautonomous transposons that typically evolve neutrally (dN/dS=0.08312 estimated using a cdNA alignment with D. yakuba). Fourth, synteny around CAG is conserved in most species of the Drosophila genus (see below) as opposed to TEs that are not expected to be maintained at orthologous positions. Fifth, CAG expresses two alternative transcripts and shows peaks of expression in different developmental stages (Marygold et al. 2013) in contrast to TE genes that are often not expressed (Deloger et al. 2009). And sixth, there are seven reported alleles for this gene, and some of them are lethal, suggesting that CAG has a critical biological function in vivo (St Pierre et al. 2013).

Thus, we can conclude that CAG has a transposon origin and it is the closest D. melanogaster host gene encoding a CR protein.

**CAG Domain Architecture Is Similar to pogo Transposase and Other CR Proteins**

We checked whether, besides sequence conservation, the domain architecture of CAG is also conserved when compared with previously identified CENP-B homologs and D. melanogaster pogo transposase (fig. 1A). Using hmmscan (Finn et al. 2011), we found that CAG shows the same composite DNA binding domain (DBD) structure found in human and S. frugiperda CR proteins (fig. 1A). The majority of the highly conserved amino acids shared by proteins having this domain are highly conserved amino acids shared by proteins having this domain structure, making it difficult to determine if they are part of the CR protein or part of the transposase domain. However, the majority of the CR proteins have a more conserved domain structure than the transposase, and the CR proteins are more conserved than the transposase.

**Fig. 1.—**Domain structure of pogo transposase and CR proteins. (A) Human CENP-B (hCENP-B), D. melanogaster pogo transposase (pogoR11), D. melanogaster CAG (CAG), yeast CENP-B homologs (Abp1, Cbh1, Cbh2), and Spodoptera frugiperda CENP-B homolog (SfCENP-B). CENP-B_N domain is shown in red, d1ufa1 in yellow, HTH, Tnp, TsC5 in green, DDE_1 in light blue, DIM in pink, and PCNA in dark blue. Domains predicted by hmmscan are shown as black-lined boxes, the other domains were inferred from experimental evidence. The discontinuous line indicates the deleted region. (B) 3D structure prediction of D. melanogaster CAG DBD using human CENP-B as a template. Z-score = −6.66 and −6.7 for CENP-B and CAG, respectively.
composite DBD are also conserved in CAG suggesting that this domain is functional (supplementary fig. S1, Supplementary Material online). Furthermore, a 3D model of CAG DBD build using human CENP-B as a template, shows that the fold of this domain is similar in both proteins (fig. 1B).

CAG is the only of the seven proteins being compared that does not have a DDE_1 endonuclease domain (DDE) next to the DBD domain (fig. 1A). Indeed, CAG is shorter than the other proteins probably due to an internal deletion, which is a common feature in this class of transposons (Negoua et al. 2013). In fact, out of the 44 pogo copies in the D. melanogaster genome, 35 showed internal deletions and one of them, F8b002096, has a similar deletion as CAG.

A dimerization domain (DIM) near the C-terminal region is present in human CENP-B (Tawaramoto et al. 2003). hmmscan does not detect any C-terminal DIM domain in the other six proteins. However, it has been described that the pogo transposase, CAG and the three yeast CENP-B homologs self-dimerize (Wang et al. 1999; Irelan et al. 2001; Giot et al. 2003; Tawaramoto et al. 2003; Cam et al. 2008; Lorenz et al. 2012). Given their common evolutionary origin, we hypothesized that the DIM domain might be located in the C-terminal region in these proteins as well. Finally, the pogo transposase has a PCNA (proliferating cell nuclear antigen) binding domain in the C-terminal end (Warbrick et al. 1998). Although this domain has not been identified in CAG at the sequence level, there is experimental evidence for CAG binding to PCNA suggesting that besides CAG other pogo-related proteins might have also conserved this function (fig. 1A).

Taken together, these results indicate that CAG is similar to the pogo transposase both at sequence and domain architecture levels, further confirming that CAG has a transposon origin. The lack of the DDE domain is explained by an internal deletion that is common in this family of transposons. Except for the absence of the DDE domain, CAG sequence and domain architecture are also similar to other described CENP-B homologs.

Protein–Protein Interaction Network Suggests CAG Is Functionally Related to Other CR Proteins

Because CAG is a protein of unknown function, we searched for proteins directly interacting with CAG to shed light on the biological processes in which this protein might be involved. Although data from protein-protein interaction (PPI) networks is still noisy and partial, functional annotation based on interaction networks provides reliable insight into the biology of proteins with unknown function (Titz et al. 2004; Sharan et al. 2007). There is experimental evidence for the interaction between CAG and 15 other proteins, and six of them have nucleic acid binding capacity (supplementary table S1, Supplementary Material online). CAG directly interacts with Mti-2, which is a component of the nucleosome remodeling and histone deacetylation (NuRD) complex with chromatin binding and remodeling activity (Bouazoune and Brehm 2005). As mentioned above, CAG has preserved the pogo transposase capacity to interact with PCNA (Warbrick et al. 1998). Besides playing a crucial role in DNA replication and repair (Warbrick et al. 1998), cell cycle control and sister chromatin cohesion (Maga and Hubscher 2003), PCNA is also a component of the microtubule associated complex (Hughes et al. 2008). CAG also interacts with Cdc5 and snama, which are involved in cell cycle control (supplementary table S1, Supplementary Material online).

To further investigate the functional annotation of CAG, we expanded the network of proteins that directly interact with CAG by incorporating their respective interaction partners (Chua et al. 2006). The resulting list of 842 proteins in the neighborhood-2 of CAG showed a significant enrichment for 72 biological process Gene Ontology (GO) terms related to cell cycle and mitotic spindle organization and nucleic acid metabolism among others (fig. 2). Fifty-eight out of the 72 CAG enriched GO terms are also enriched in the human CENP-B neighborhood-2, further suggesting that CAG and CENP-B interacting partners are involved in similar biological processes.

CR Genes Are Present in Holocentric and Nonholocentric Insecta

To determine whether CAG is present in species other than D. melanogaster, we searched for CAG orthologs using a BLASTp search against ensembl metazoa protein database (see Materials and Methods). CAG has nine 1-to-1 orthologs in the Drosophila genus that showed a sequence identity from 97.2% to 39.8% (table 1). The DBD architecture is conserved in all of them and the length of the protein is highly similar except in D. simulans, where only the first DBD domain is present (table 1). Other than sequence identity and protein length, synteny is also conserved in the six closest Drosophila species except in D. simulans. Furthermore, CAG is evolving under functional constrain in these ten Drosophila species (average difference of synonymous and nonsynonymous substitutions per site over all nine sequence pairs is 17.99, P value ≪ 0.001) suggesting that CAG orthologs are functional genes.

Other than in the Drosophila genus, CAG has homologs in four Lepidoptera species and in one Coleoptera with sequence identities ranging from 51.5% to 22.2% (table 1). We could only detect TIRs flanking Heliconius melpomene HMEL010729 suggesting that the other identified homologs are not transposons but transposon-derived genes (see Materials and Methods).

Overall, our results show that CR genes are present both in holocentric, Lepidoptera, and in nonholocentric, Diptera and Coleoptera, species.
CAG Belongs to the CR Clade

We constructed a phylogenetic tree to find out where insect CENP-B homologs are located in the previously published phylogeny containing a representative set of pogo transposases and pogo-derived genes (Casola et al. 2008). Phylogenetic trees of the full sequence set containing nonmetazoan transposases and transposase-derived genes can be found in supplementary figures S2 and S3, Supplementary Material online (see Materials and Methods). Our tree recovers the two monophyletic clades in metazoans: CR and Jerky related (JR) (fig. 3). CAG is located in the CR clade, and as expected, its closest transposase is the *D. melanogaster* pogo. The closest non-*Drosophila* CAG homolog is *Tribolium castaneum* TC005011. Most of the other insect CENP-B homolog genes, including the already described *S. frugiperda* and *H. armigera* CENP-B homologs, also fell in the CR clade. Insect and mammalian CR proteins form subclades inside the CR clade (fig. 3). Other than between *D. melanogaster* CAG and *D. ananassae* GF13390 transposase-derived genes, synteny is also conserved among Sfru_72F01, Harmi_94B11_25, and Bombyx_BGIBMGA013624 suggesting that at least two additional independent exaptations, besides the mammal and fission yeast exaptations reported by Casola et al. (2008), have occurred.

Note that *Helicon. melpomene* homologs form two clusters, one in the JR clade and one in the CR clade, showing extensive sequence identity indicating that they are...
either recent duplications or miss-annotated transposons (fig. 3).

**Pogo-like Transposases Have Been Recurrently Exapted into CR Proteins in Metazoans**

In this work, we have identified CAG as the closest CR protein in the *D. melanogaster* genome. Similar to other CR proteins, CAG has originated from the domestication of a pogo transposase and might be functionally related to other CENP-B homologs as suggested by the conservation of three out of the four functional domains (fig. 1) and the GO enrichment analyses of CAG PPI network (fig. 2). Knowledge about the contribution of each particular domain to the overall functions of CR proteins is scarce (Okada et al. 2007; Lorenz et al. 2012). However, conservation of DBD domain appears to be particularly important because it has been demonstrated that binding of this domain is sufficient to promote chromatin assembly in humans (Okada et al. 2007). Both sequence identity and 3D structure prediction show that CAG has a highly conserved DBD domain (fig. 1).

Other than in *D. melanogaster*, we were also able to identify CR proteins in *T. castaneum*, which is also a nonholocentric insect, indicating that CR proteins are not restricted to holocentric insecta (table 1) (d’Alenc¸on et al 2011). Insect CENP-B homologs do not form a single monophyletic clade: Most sequences are part of the CR clade and a few belong to the JR clade. Furthermore, insect and mammalian CR proteins form moderately supported subclades inside the CR clade (fig. 3). These results suggest that at least three independent domestications of pogo-like transposases into CR proteins have occurred in metazoans (fig. 3).

**Table 1**

| Class     | Order   | Species              | Protein Identifier* | Protein Sequence Identityb (%) | Protein Length | Conserved Protein Domains |
|-----------|---------|----------------------|---------------------|---------------------------------|----------------|---------------------------|
| Insecta   | Diptera | *Drosophila melanogaster* | CAG (CG12346)      | 100                             | 225            | X                         |
|           |         | *D. simulans*        | GD15259             | 97.22                           | 111            | X                         |
|           |         | *D. sechelia*        | GM20484             | 94.67                           | 225            | X                         |
|           |         | *D. yakuba*          | GE13064             | 92.44                           | 225            | X                         |
|           |         | *D. erecta*          | GG22708             | 93.24                           | 207            | X                         |
|           |         | *D. ananassae*       | GF13390             | 59.11                           | 222            | X                         |
|           |         | *D. pseudoobscura*   | GA11571             | 57.46                           | 228            | X                         |
|           |         | *D. persimilis*      | GL17090             | 58.77                           | 228            | X                         |
|           |         | *D. willistoni*      | GK19073             | 40.29                           | 222            | X                         |
|           |         | *D. virilis*         | GJ16124             | 39.81                           | 227            | X                         |
| Insecta   | Lepidoptera | *Bombyx mori* | BGIBMGA013031      | 32.09                           | 278            | X                         |
|           |         | BGIBMGA008012        | 26.35               | 501                             | X             |
|           |         | BGIBMGA007903        | 25                  | 468                             | X             |
|           |         | BGIBMGA013624        | 29.63               | 722                             | X             |
| Insecta   | Lepidoptera | *Heliconius melpomene* | HMEL009793       | 35.38                           | 255            | X                         |
|           |         | HMEL010729           | 33.8                | 295                             | X             |
|           |         | HMEL014790           | 31.55               | 533                             | X             |
|           |         | HMEL007960           | 50                  | 533                             | X             |
|           |         | HMEL011593           | 35.38               | 192                             | X             |
| Insecta   | Lepidoptera | *Helicoverpa armigera* | 94B111.25*         | 22.22a                          | 488            | X                         |
|           |         | 72F01*               | 23.61a              | 488                             | X             |
| Insecta   | Coleoptera | *Tribolium castaneum* | TC003750           | 26.39                           | 1175           | X                         |
|           |         | TC001653             | 30                  | 486                             | X             |
|           |         | TC005011             | 51.49               | 212                             | —             |

*All sequences can be downloaded from Ensembl Metazoa except those with an "**" that can be downloaded from LepidoDB.

bProtein sequence identity estimated using BLASTp except for those with an "***" estimated using ClustalW (see Materials and Methods).
Phylogenetic distribution of pogo-related transposases and transposase-derived genes in metazoans. JR and CR indicate that the sequences belong to the JR clade and the CR clades, respectively. Filled-boxes depict pogo-related transposases and empty boxes depict transposase-derived genes. Numbers in the nodes show posterior probabilities (black) and bootstrap values (red). Shaded branches correspond to new CR proteins identified in this work and in d’Alenc¸o ne ta l 2 011 (table 1) that have been incorporated to the previously published phylogeny (Casola et al 2008). Dotted lines represent branches not drawn to scale. Trees including nonmetazoans pogo-related transposases and transposase-derived genes are depicted in supplementary figures S2 and S3, Supplementary Material online.
and other DNA binding proteins (supplementary table S1, Supplementary Material online).

Overall, our results suggest that the numerous TE exaptations already described might just be the tip of the iceberg, and highlight the role of TEs as raw material for the recurrent evolution of important cellular functions (Bowen and Jordan 2007; Sinzelle et al. 2009).

Materials and Methods

CAG Identification

Human CENP-B (P07199), fission yeast Abp1 (NP_596460), Cbh1 (CAB16408), Cbh2 (CAA19330.1), S. frugiperda CENP-B (72F01) (d’Alençon et al. 2011), and pogo transposase (S20478) were used as BLASTp queries against D. melanogaster nr protein database with BLOSUM45 scoring matrix, low-complexity region filter, and an e value cutoff of 1e-04. Significant hits were used in a reciprocal BLASTp search using these same parameters.

Domain Architecture Analysis

Hmmmscan software implemented at HMMER 3.1b1 (Finn et al. 2011) was used to search for occurrences of the domains deposited in Pfam-A database in the protein sequences under study. This information was complemented with the structural data available for human CENP-B (PDBIDs: 1HLV, 1BW6, 1UFI) and fission yeast Abp-1 (PDBID: 1UFI). The experimentally determined molecular interactions reported by Warbrick et al. (1998), Giot et al. (2003), Guruharsha et al. (2011), and Irelan et al. (2001) that were accessed through the PSICQUIC web server (Aranda et al. 2011) and FlyBase (Marygold et al. 2013) were also taken into account.

CAG 3D Modeling

The crystal structure of human CENP-B DBD (PDB ID: 1HLV) was used as a template for the modeling of D. melanogaster CAG DBD. The first 145 aminoacids (aa) of CAG were aligned to the 131 aa contained in the crystalized human CENP-B DBD using a combination of a global alignment built with ClustalW and two local alignments of the HTH regions built with hmmalign software (Finn et al. 2011) and the pfam profiles CENP-B_N and HTH_Tnp_Tc5. The resulting alignment was manually refined taking into account the predicted secondary structure of CAG and the description of the secondary structure of the crystal. Several alignments were used as an input to build CAG DBD models using the automodel class in Modeller 9.7 (Eswar et al. 2007). The resulting models were evaluated taking into account their stereochemical properties calculated by PROCHECK (Laskowski et al. 1993) and their pseudoenergetic profiles and z-scores calculated by PROSA-II (Wiederstein and Sippl 2007). STAMP was used to visualize the superimposition of the models with human CENP-B (Russell and Barton 1992).

CAG PPI Network

A list of proteins with experimental evidence of direct interaction with CAG (Q7JR24) and human CENP-B (P07199) were retrieved from Drosophila Protein Interaction Map and the PSICQUIC web server (Aranda et al. 2011). Only those binary interactions obtained using experimental detection methods and interaction types “association”, “physical association,” or “direct interaction” were kept.

The PPI interaction network at neighborhood-1 of CAG and CENP-B was expanded to neighborhood-2 using both experimentally determined and predicted interactions deposited at Interolog Finder web server (Wiles et al. 2010). We retrieved a list of 1,413 interactions involving 842 proteins for CAG and a list of 1,665 interactions involving 1,174 proteins for CENP-B. Cytoscape was used to visualize the interactions and BinGO to assess the overrepresentation of GO terms (Maere et al. 2005). Those GO terms showing a Benjamin and Hochberg False Discovery Rate corrected P value <1e-10 in a hypogeometric statistical test were represented, together with their parent terms, in a hierarchical layout. The intersection of the GO terms that were enriched in both CAG and CENP-B neighborhood was performed by comparing the generated output files.

Codon-Based Test of Purifying Selection

Coding sequences for nine CAG orthologs in Drosophila species were aligned by ClustalW (Thompson et al. 1994). Codon-based tests of selection analyses were conducted in MEGA5 (Hall 2013) using the Nei–Gojobori method (Nei and Gojobori 1986). All ambiguous positions were removed for each sequence pair. The average difference of synonymous and nonsynonymous substitutions per site was calculated. The variance of the difference was computed using the bootstrap method (500 replicates).

Identification of CAG Orthologs and CR Genes

BLASTp searches using CAG sequence as a query were performed against ensembl metazoa protein databases. Protein sequences of those hits showing an E value smaller than 10^-4 and a protein sequence identity greater than 25% along at least 100 aa, were retrieved and used for further analyses. We then checked whether the identified proteins were reported as orthologs in ensembl metazoa compara, Genomicus, and OrthoDB Arthropods, and kept only the ones that were reported as orthologs in at least one of the three databases. The two previously described CR genes in S. frugiperda and H. armigera were also included in the analyses (d’Alençon et al. 2011). For these two sequences percentage of protein sequence identity was estimated using ClustalW. We then confirmed that the sequences had not been annotated as TEs during their respective genome annotation projects (Tribolium Genome Sequencing Consortium 2008; Duan et al. 2010; Heliconius Genome Consortium 2012). To further
confirm that these sequences correspond to host proteins and not to transposases, we searched for Terminal Inverted Repeats in the 5’ and 3’ 600 base-pair regions flanking the CDS. To this end, we performed local sequence alignments using the Smith–Waterman algorithm for all possible sliding windows of 24 bp in the 5’-flanking region and the reverse sequence of the 3’-flanking region. *Drosophila melanogaster PogoR11* was used as a positive control.

**Phylogenetic Analysis of pogo-Related Sequences**

Global multiple sequence alignments were performed using *MAFFT (L-INS-I algorithm)* (Katoh and Standley 2013). Local alignment of the DBD was performed using hmalign and the hidden markov models *HTH_CENP-B_N* and *HTH_Tnp_Tc5*. Both alignments were combined and manually curated to obtain a final multiple sequence alignment of 449 residues with a proportion of gaps of 15.90%. This alignment was used to reconstruct the phylogeny of the *pogo*-related transposases and transposase-derived genes. We estimated the maximum-likelihood (ML) tree using *RAxML* (Stamatakis 2014). We used the best-fit amino acid substitution matrix *LG* empirical mixture model with a discrete gamma distribution with four categories where constant sites were removed. Two Markov chains were run in parallel with a burn-in of 1000000 and 100 bootstrap replicates were performed (supplementary fig. S2, Supplementary Material online). Because bootstrap support in most of the branches in the metazoan sequences were smaller than 70, we decided to perform an independent inference using a Bayesian approach. We constructed the phylogenetic tree with PhyloBayes using the LG empirical mixture model with a discrete gamma distribution with four categories where constant sites were removed. Two Markov chains were run in parallel with a subsampling frequency of 100 until convergence was reached (population effective size of 242, maximum difference of 0.105175, mean difference of 0.00381156) (supplementary fig. S3, Supplementary Material online). ETE Toolkit (Huerta-Cepas et al. 2010) was used to annotate and visualize phylogenetic trees.

Genomicus (Louis et al. 2013) was used to check for synteny conservation in the different subclades identified.

**Supplementary Material**

Supplementary table S1 and figures S1–S3 are available at Genome Biology and Evolution online (http://www.gbe.oxfordjournals.org).

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