



HAL
open science

Repeated horizontal gene transfers triggered parallel evolution of magnetotaxis in two evolutionary divergent lineages of magnetotactic bacteria

Caroline Monteil, Denis Grouzdev, Guy Perriere, Béatrice Alonso, Zoé Rouy, Stéphane Cruveiller, Nicolas Ginet, David Pignol, Christopher T. Lefèvre

► To cite this version:

Caroline Monteil, Denis Grouzdev, Guy Perriere, Béatrice Alonso, Zoé Rouy, et al.. Repeated horizontal gene transfers triggered parallel evolution of magnetotaxis in two evolutionary divergent lineages of magnetotactic bacteria. *The International Society of Microbiological Ecology Journal*, 2020, 10.1038/s41396-020-0647-x . hal-02625381

HAL Id: hal-02625381

<https://cnrs.hal.science/hal-02625381>

Submitted on 7 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Repeated horizontal gene transfers triggered parallel evolution of**
2 **magnetotaxis in two evolutionary divergent lineages of magnetotactic bacteria**

3
4 Caroline L. Monteil^{1,*}, Denis S. Grouzdev², Guy Perrière³, Béatrice Alonso¹, Zoé Rouy⁴,
5 Stéphane Cruveiller⁴, Nicolas Ginet⁵, David Pignol¹ & Christopher T. Lefevre^{1*}

6
7 ¹Aix-Marseille Univ, CEA, CNRS, Biosciences and Biotechnologies Institute of Aix-Marseille, Saint Paul lez
8 Durance, France.

9 ²Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow,
10 Russia

11 ³Laboratoire de Biométrie et Biologie Evolutive, CNRS, UMR5558, Université Claude Bernard – Lyon 1,
12 69622, Villeurbanne, France

13 ⁴LABGeM, Génomique Métabolique, CEA, Genoscope, Institut François Jacob, CNRS, Université d'Évry,
14 Université Paris-Saclay, Evry, France

15 ⁵Aix Marseille University, CNRS, LCB, Marseille, France

16

17

18

19

20

21

22 *Correspondence: Caroline Monteil, c.l.monteil@hotmail.com and Christopher Lefevre,
23 christopher.lefevre@cea.fr

24

25 Keywords: parallel evolution, microbial adaptation, anoxic sediments, horizontal gene
26 transfer, mutation, magnetotaxis, bacteria

27 **Abstract**

28 Under the same selection pressures, two genetically divergent populations may evolve in
29 parallel towards the same adaptive solutions. Here, we hypothesized that magnetotaxis (i.e.
30 magnetically guided chemotaxis) represents a key adaptation to micro-oxic habitats in
31 aquatic sediments and that its parallel evolution homogenised the phenotypes of two
32 evolutionary divergent clusters of freshwater spirilla. All magnetotactic bacteria affiliated
33 to the *Magnetospirillum* genus (Alphaproteobacteria class) biomineralize the same
34 magnetic particle chains and share highly similar physiological and ultrastructural features.
35 We looked for the processes that could have contributed at shaping such an evolutionary
36 pattern by reconciling species and gene trees using newly sequenced genomes of
37 *Magnetospirillum* related bacteria. We showed that repeated horizontal gene transfers and
38 homologous recombination of entire operons contributed to the parallel evolution of
39 magnetotaxis. We propose that such processes could represent a more parsimonious and
40 rapid solution for adaptation compared to independent and repeated *de novo* mutations,
41 especially in the case of traits as complex as magnetotaxis involving tens of interacting
42 proteins. Besides strengthening the idea about the importance of such a function in micro-
43 oxic habitats, these results reinforce previous observations in experimental evolution
44 suggesting that gene flow could alleviate clonal interference and speed up adaptation under
45 some circumstances.

46

47 **Introduction**

48 Magnetotaxis couples the biomineralization of ferrimagnetic nanoparticles in organelles
49 called magnetosomes, to a complex system of aero-chemotaxis for guiding the locomotion
50 of magnetotactic bacteria (MTB) parallel to the Earth's magnetic field lines [1]. This
51 function is particularly well described in a group of freshwater MTB of the
52 Alphaproteobacteria class represented by the *Magnetospirillum* genus (MTB_{Mag}).
53 Magnetotaxis is assumed to facilitate vertical navigation towards their optimal niches
54 located at the oxic-anoxic transition zone [2]. Our knowledge of the genetic basis, ecology
55 and biophysical processes involved in magnetite magnetosome biogenesis was built mainly
56 from the study of two model strains, *Magnetospirillum gryphiswaldense* strain MSR-1 [3]
57 and *Magnetospirillum magneticum* strain AMB-1 [4–6]. Since their first observation,
58 numerous other spirilla were isolated and affiliated to the same group based on phenotypic,
59 physiological and morphological features, among which the ability to form magnetosomes
60 [7–11]. As for many prokaryotes, the increasing biodiversity assessment and the
61 development of molecular typing revealed the polyphyletism of magnetotaxis in freshwater
62 Rhodospirillaceae (Alphaproteobacteria) [8, 12]. Members of two genera with different
63 lifestyles: *Phaeospirillum* and *Dechlorospirillum* are actually more closely related to some
64 *Magnetospirillum* lineages than what some *Magnetospirillum* lineages are to each other [7,
65 8, 12, 13]. The genus *Phaeospirillum* contains spiral-shaped, phototrophic, purple
66 nonsulphur bacterial species [14], while *Dechlorospirillum*, now affiliated to the
67 *Magnetospirillum* genus based on phylogenetic analyses, is represented by non-MTB only
68 [15, 16].

69 The polyphyletism of magnetotaxis and the lack of a clear correlation between
70 genetic clusters and ecology created some confusion in the classification of these organisms
71 that remains partially resolved [17]. Today, we are still unable to state whether or not
72 magnetotactic *Magnetospirillum* form their own genera [8, 18] because their specific
73 ecological boundaries have not been identified yet and they do not form a monophyletic
74 group [8, 12]. Current data support the existence of two genetically distinct groups

75 represented by strains MSR-1 and AMB-1 respectively [8]. All magnetotactic strains
76 affiliated to the *Magnetospirillum* genus fix carbon dioxide through the Calvin-Benson-
77 Bassham (CBB) cycle with the presence of a form II ribulose-1,5-bisphosphate
78 carboxylase/oxygenase (RuBisCO) gene in their genome [8, 19], and all seem to fix
79 atmospheric nitrogen [20, 21]. Other shared features of described magnetotactic
80 *Magnetospirillum* species relate to biomineralization. They form a single chain of
81 biomineralized cuboctahedral crystals of magnetite, have a bipolarly flagellated helical
82 shape and a respiratory form of metabolism that uses organic acids as a source of carbon and
83 electrons [3]. Moreover, the magnetite synthesis occurs only at very low levels of oxygen
84 or under anaerobic conditions when nitrate is the alternative terminal electron acceptor to
85 oxygen [22–25]. Their metabolic specificities are less obvious to pinpoint, maybe because
86 some traits are variable within species or because they were insufficiently characterized. It
87 seems that their requirement for energy source and carbon source may vary according to
88 the strain. For example, while most species are facultative anaerobes that utilize nitrate as
89 an alternative terminal electron acceptor to oxygen, *M. magnetotacticum* is an obligate
90 microaerophile that requires oxygen even when growing with nitrate [22] and *M.*
91 *gryphiswaldense* can grow autotrophically using reduced sulphur compounds as electron
92 donor [26].

93 This high degree of phenotypic homogeneity of magnetic spirilla suggests an adaptation to
94 similar environments that exert important constraints on their metabolism and
95 magnetotaxis. From an evolutionary perspective, the common genetic determinism to all
96 MTB indicates a single emergence of magnetotaxis in prokaryotes. Magnetosome biogenesis
97 is encoded by unique genes (referred as the *mam* and/or *mms* genes) that are, for most of
98 them, clustered into operons within a specific genomic region [27–29]. Compiling studies
99 show both evidence of vertical inheritance of these genes [12, 30, 31] and multiple
100 acquisitions by horizontal gene transfer (HGT) in some lineages in MTB of the
101 Alphaproteobacteria class [32–34] with duplication events [34, 35]. These observations raise
102 questions on the evolutionary mechanisms shaping the adaptation to this ecological niche

103 and the phenotypic maintenance at such level of phylogenetic distance between two
104 evolutionarily distinct lineages.

105 Selection contributes to divergent evolution and different ecological speciation in the
106 presence of reproductive isolation [36]. However, similar environments in different
107 locations may promote parallel or convergent evolution of traits and maintain a high degree
108 of phenotypic similarity in independent and divergent populations [37]. Tracking the
109 genetic parallelism involved in parallel ecological speciation may help to better understand
110 the mechanisms of adaptive evolution. Because similar niches may exert the same degree of
111 evolutionary constraint on traits, the same *de novo* beneficial mutations are likely to be
112 repeatedly selected and fixed in populations [37–39]. In microorganisms, other evolutionary
113 forces than mutation, like homologous recombination or orthologous replacement
114 mediated by HGT generate genetic variation [40–42]. In prokaryotes, the contribution of
115 these mechanisms in parallel evolution specifically has been less documented [43], although
116 HGT and homologous recombination could promote the repeated exchange of beneficial
117 alleles in genetically distant organisms, purge the genic variability to maintain phenotypic
118 and ecological cohesiveness in an ecotype [44]. In laboratory-controlled experiments,
119 recombination has been shown to alleviate clonal interference and accelerate adaptation of
120 *Escherichia coli* populations under some circumstances [45]. *In natura*, two divergent
121 populations inhabiting the same habitat are more likely to exhibit weaker barrier to gene
122 flow than do different ecotypes, regardless the genetic distance [42, 46]. Our understanding
123 of these processes leading to ecological differentiation in bacteria suffers from the lack of
124 niches and habitats studied. Indeed, our vision has been built mainly from studies on
125 pathogens [44]. But a broader investigation of environmental niches and ecotypes may help
126 to map bacterial diversity onto ecology involving other traits than those related to cell
127 surface, DNA binding and pathogenicity-related functions [47].

128 Here, we investigated the evolutionary mechanisms involved in the parallel
129 evolution of magnetotaxis in two distinct groups of magnetotactic *Magnetospirillum*
130 species. We particularly tested the hypothesis that recombination *sensus lato* could be
131 involved in the maintenance of highly homogenous phenotypes in two divergent lineages

132 keeping them adapted to very similar niches. We studied the biology and genome of newly
133 isolated freshwater magnetotactic and non-magnetotactic *Magnetospirillum* related
134 species. By comparing gene contents and inferring phylogenies based on whole genomes
135 and magnetosomes associated genes, we showed that ancestors of these groups exchanged
136 their magnetosome gene clusters several times over the course of magnetotactic
137 *Magnetospirillum* diversification, which might have been at the origin of the maintenance
138 of a high degree of global genomic conservation and phenotypic similarity. Our results
139 showed that repeated gene exchanges of entire operons involved in sensing and motility
140 may contribute to the parallel evolution of species facing similar environmental constraints,
141 which likely accelerate their adaptation to microoxic conditions.

142

143 **Materials and methods**144 **Genomes collection and growth of bacteria**

145 Draft and closed genomes of all Rhodospirillaceae available in October 2017 were
146 downloaded from the public repository database at NCBI and uploaded into the MicroScope
147 platform for their automatic and manual annotation [48]. Sequencing and assembling of
148 *Magnetospirillum magneticum* AMB-1 [49], *M. gryphiswaldense* MSR-1 [50], *M.*
149 *magnetotacticum* MS-1 [51], *M. caucaseum* SO-1 [52], *M. marisnigri* SP-1, *M. aberrantis*
150 SpK [53], *M. bellicus* VDY [54], *Magnetospirillum* sp. XM-1 [55], *Magnetospirillum* sp.
151 ME-1 [56], *P. fulvum* MGU-K5 [57], and *P. molischianum* DSM120 [58] were described
152 previously. Draft genome assemblies of non-magnetotactic *Magnetospirillum* strains CP2,
153 CP3, VDY and WD, and those of *Phaeospirillum* strains DSM 114, DSM 115, DSM 117 and
154 DSM 13234 were provided by the Joint Genome Institute (www.jgi.doe.gov) and described
155 in supplementary **Table S1**. This list was completed with 5 genomes sequenced in this study.
156 Genomes of magnetotactic strains LM-1, SS-4, UT-4, LM-5, and CP-1 were sequenced and
157 assembled following the exact procedure described in [34] based on genomic DNA obtained
158 from axenic cultures. Their sampling sites, the isolation procedure and culture medium
159 were described in [8], with the exception of strain CP-1 reported here for the first time.
160 Strain CP-1 was isolated using a similar procedure as previously described [8], from the
161 freshwater river Couze Pavin, Auvergne, France (45°30'35.0"N, 2°54'12.6"E).
162 Chemolithoautotrophic growth experiments were carried out on strains AMB-1, SS-4, CP-
163 1, MSR-1, UT-4, LM-1 and LM-5 using a similar semi-solid growth medium as previously
164 described [8] but removing sodium acetate and sodium sulphide and adding after autoclave
165 3 ml of 40 % sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$). Thiosulfate was deemed positive for the
166 support of growth if three conditions were met: (i) a band of cells formed in the tube, (ii)
167 the band after growth was significantly thicker than the control containing no thiosulfate
168 and (iii) cells continued to grow in the same medium in three successive transfers. The draft
169 genome sequences partially annotated of LM-1, SS-4, UT-4, LM-5, and CP-1 were

170 submitted to the European Nucleotide Archive and carry the accession numbers
171 PRJEB35448, PRJEB35447, PRJEB35446, PRJEB35445 and PRJEB35444, respectively.

172 **Light and electron microscopy**

173 Motility and magnetotactic behaviour of the different *Magnetospirillum* strains were
174 analysed and recorded under the light microscope Leica LMD6000 equipped with the
175 camera Leica DMC 4500. Transmission electron microscopes (TEM) was used to analyse the
176 ultrastructure of *Magnetospirillum* strains directly deposited onto TEM copper grids coated
177 with a carbon film. Magnetosomes shape and organization were visualized with a Tecnai
178 G2 BioTWIN (FEI Company, Eindhoven, Netherlands) equipped with a CCD camera
179 (Megaview III, Olympus Soft imaging Solutions GmbH, Münster, Germany) with an
180 accelerating voltage of 100 kV.

181 **Comparative genomics**

182 Evolutionary relationships between *Magnetospirillum*-related species and their closest
183 members of the Rhodospirillaceae family were investigated using whole genome sequences.
184 Clusters of orthologous were defined using the OrthoMCL clustering algorithm
185 implemented in GET_HOMOLOGUES open-source software [59]. We considered any
186 cluster of orthologous proteins for which pairwise BLASTP alignments had an expectation
187 value $E < 10^{-5}$ and a minimal protein coverage of 50%.

188 Genomes and encoded proteomes of *Magnetospirillum* species and their closest relatives
189 were compared with several complementary algorithms to define sub-groups/species based
190 on their nucleotidic/amino acid composition : (i) Average Nucleotide Identity (ANI) and
191 Average Amino acid Identity (AAI) values were calculated using the ANI/AAI-Matrix
192 online service (<http://enve-omics.ce.gatech.edu/g-matrix/>) [60] using reciprocal best hits
193 (<http://enve-omics.ce.gatech.edu/>), (ii) digital DNA-DNA hybridization (dDDH) values
194 were determined using the Genome-to-Genome Distance Calculator (GGDC 2.1;
195 <https://ggdc-test.dsmz.de/ggdc.php>) using the recommended BLAST+ alignment and

196 formula 2 (identities/HSP length) [61] and (iii), percentages of conserved proteins (POCP)
197 [62] were calculated using the script runPOCP.sh [63].

198 Metabolic pathways were predicted with the Microscope platform [64] using the Pathologic
199 algorithm [65], which computes an initial set of pathways by comparing genome
200 annotations to a collection of microbial Pathway/Genome Databases (PGDBs) MicroCyc
201 derived from the metabolic reference database MetaCyc [66]. For each MicroCyc pathway,
202 the tool gives the completion value referring to the number of reactions in a given
203 strain/total number of reactions in the same pathway defined in the MetaCyc database.

204 The presence of conjugative system was checked in all genomes using MacSyFinder
205 software and the TXSScan module [67, 68], which uses HMM profiles for efficient genomic
206 detection of bacterial secretion systems encountered in diderm-LPS bacteria, and their
207 discrimination from homologous machineries.

208 **Statistical analyses**

209 Statistical analyses were performed in R version 3.5.1 [69]. Averages of AAI and POCP
210 values estimated from the pairwise comparison of genomes sets were compared either by
211 the Student's t-test or by the non-parametric Mann–Whitney U test (if the underlying
212 assumptions of the first test were not satisfied). Differences were considered significant
213 when P-values were below 0.05.

214 A principal component analysis (PCA) was performed using the ade4 package [70] to
215 compare metabolic pathways of the 23 *Magnetospirillum* relatives using a reduced matrix
216 of MicroCyc pathway completion values generated with the MicroScope platform. The
217 reduced matrix consisted of the 99 pathways whose completion was variable between
218 groups among the 410 non-redundant pathways detected in at least one genome.

219 **Phylogenetic analyses**

220 For phylogenetic analyses, only clusters with single-copy sequences were taken into
221 consideration and in-paralogs were excluded. All complete gene outliers were removed

222 with Phylo-MCOA software [71]. For the species tree, amino acids sequences of 839
223 orthologous proteins were aligned independently using MAFFT [72] and alignments were
224 trimmed using BMGE [73] setting block length to 3, and concatenated into a final alignment
225 of 269,968 amino acids positions among which 151,377 were polymorphic. The maximum
226 likelihood tree was built from the concatenated sequences with IQ-TREE [74] using a
227 partition model; the substitution model for each protein was selected by ModelFinder [75]
228 with the Bayesian Information Criterion (BIC). The statistical support of the branches was
229 estimated by the ultrafast bootstrapping approach implemented in IQ-TREE applying 1000
230 replicates. A previous study suggesting that strain LM-1 was a new freshwater *MTB_{Mag}*
231 genus [8], we included this strain in the external group for phylogenetics and comparative
232 genomics.

233 Evolution of genes encoding for proteins involved in magnetosome biogenesis was studied
234 by reconciling binary gene trees built from gene-by-gene alignments of *mam* genes or their
235 concatenation with the non-binary species tree under the DTL parsimony algorithm
236 implemented in Notung 2.9 software [76]. The algorithm captures gene duplication (D),
237 transfer (T) and loss (L) driving tree incongruence and infers all optimal solutions to finally
238 report the complete and temporally feasible event histories giving the data.

239

240 **Results**241 **Species of magnetotactic *Magnetospirillum* do not form a monophyletic group at the whole**
242 **genome level**

243 We built a phylogenetic framework to study the evolution of magnetotaxis in MTB_{Mag} at
244 the whole genome scale using draft genomes of freshwater magnetotactic and non-
245 magnetotactic bacteria close to *Magnetospirillum gryphiswaldense* MSR-1 or *M.*
246 *magneticum* AMB-1 (Table S1). A first phylogenetic tree based on all proteins shared by
247 strains representing the Rhodospirillaceae diversity and related other Alphaproteobacteria
248 confirmed that all 23 *Magnetospirillum* relatives in this study cluster together at the
249 genome level and positioned strain LM-1 as the closest freshwater strain of this group (Fig.
250 S1). Based on this phylogeny and what we currently know about the biology of strain LM-
251 1 [8], this strain represents a new genus. Whereas *Magnetospirillum* species exhibit a
252 spirillum shape with flagella at both poles and synthesize cuboctahedral crystals, strain LM-
253 1 displays a vibrio shape with a single polar flagellum and synthesizes elongated prismatic
254 crystals resembling those of *Magnetovibrio blakemorei* strain MV-1 [8]. Despite these
255 morphological similarities between MV-1 and LM-1, the proposed LM-1 genus is much
256 closer to freshwater Rhodospirillaceae MTB than to marine Rhodospirillaceae MTB. Thus,
257 we propose the name *Candidatus* Magneticavibrio boulderlitore (the magnetic vibrio
258 isolated from the shore of boulder beach in Lake Mead) [8]. We further used strain LM-1
259 as a control and external outgroup to *Magnetospirillum* related taxa in this study.

260 The phylogeny of *Magnetospirillum* related species only was then reconstructed based on
261 839 orthologous proteins selected after all complete gene outliers were removed [71]. The
262 tree topology was partially congruent with those of the trees built from 16Sr RNA sequences
263 of some strains in previous studies [8, 17]. For example here, the strain UT-4 clusters with
264 the MSR-1 clade (Fig. 1A), while it was supposed to be ancestral to all MTB_{Mag}. The strain
265 CP-1, isolated here from the Couze Pavin River, France, clusters with the ancestor of all
266 AMB-1 related strains. Together, all *Magnetospirillum* related species form four

267 monophyletic groups (namely SpK, MSR-1, MGU-K5 and AMB-1 clades), among which
268 two: MSR-1 and AMB-1, are composed of magnetotactic bacteria only. The non-
269 magnetotactic MGU-K5 clade (*Phaeospirillum* species) shares a more recent ancestor with
270 the AMB-1 clade composed of strains ME-1, XM-1, SO-1, AMB-1, SS-4, SP-1 and CP-1. The
271 SpK clade (formerly *Dechlorospirillum* including *M. aberrantis*) is composed of non-
272 magnetotactic bacteria only (CP2, CP3, VDY, WD and SpK) and shares a more recent
273 ancestor with the MSR-1 clade composed of magnetotactic strains LM-5, BB-1, MSR-1 and
274 UT-4. These two polyphyletic groups regarding magnetotaxis relate to each other with a
275 direct common ancestor.

276 **Lifestyle and environment maintain genetic relatedness and phenotypic homogeneity in** 277 **two divergent magnetotactic lineages**

278 The intriguing polyphyletism of magnetotaxis in MTB_{Mag} and the high degree of phenotypic
279 divergence of species of the MGU-K5 and SpK clades raised questions about the definition
280 of *Magnetospirillum* as a genus. By calculating several indexes that are generally used to
281 refine taxonomic boundaries for prokaryotes like the AAI, ANI, dDDH or POCP, we
282 investigated the support of their classification at the genome level (Fig. S2 to S5). The
283 comparison of genomes pairwise within and between monophyletic groups confirmed the
284 existence of four clades for which AAI values ranged from 64% to 70% (Fig. 1c). These
285 values justify a new genus description for each group according to previous large metadata
286 analyses assuming a significant ecological differentiation [60]. Here, it was interesting to
287 observe higher POCP values between all magnetotactic species than between these species
288 and their genetically closer non-magnetotactic species, which showed that MTB lifestyle
289 fostered the maintenance of similar genome contents (Fig. 1c). On the basis of their
290 phenotype, different magnetotactic species studied here and previously were difficult to
291 distinguish using light and electron microscopy approaches [3, 4, 7–11, 26, 77]; they share
292 all very similar magnetotactic behaviours, motilities, cell ultrastructures and magnetosome
293 morphology/organization (Fig. S6). Experimental attempts to find a different physiology
294 specific to one of the two groups failed too. According to the literature, chemolithoautophy

295 with thiosulfate as electron donor was potentially the only difference between AMB-1 and
296 MSR-1 [26].

297 We tested this hypothesis *in silico* by comparing whole genomes and noticed that some
298 genes involved in the thiosulfate-oxidizing Sox enzyme system were specific to the MSR-1
299 clade (Table S2). Testing chemolithoautotrophy with thiosulfate as electron donor for
300 strains of both clades, we showed that not all members of the MSR-1 group, such as strain
301 LM-5, were able to use thiosulfate as energy source, while none of the cultured species
302 tested of the AMB-1 group grow in such conditions. No other clear interpretation could be
303 made about the biological differences between magnetotactic *Magnetospirillum* lineages.
304 We thus looked for other genomic evidences of clear biological boundaries by
305 reconstructing the metabolic pathways. Principal component analysis (PCA) built from the
306 matrix of pathway complementation (Fig. 1d, Table S3), confirmed that *Phaeospirillum*
307 species do not cluster with the other lineages and that AMB-1 clade is metabolically closer
308 to that of MSR-1, than MGU-K5 and SpK clades. Searching pathways that participate the
309 most to the variability for both PCA axis, we confirmed that MGU-K5 relatives use light-
310 harvesting complexes with the impossibility to denitrify, degrade alanine or produce
311 vitamin B₁₂. Differences between the three other groups were much less significant and
312 were associated, for example, to creatinine degradation, cyclopropane fatty acids,
313 spermidine and preQ0 metabolite biosynthesis. In the absence of significant ecological
314 differentiation for the non-photosynthetic clades, there is no support for the creation of
315 two genera for AMB-1 and MSR-1 clades.

316 **Conservation of *mam* genes composition and synteny supports magnetotaxis was**
317 **functionally constrained by similar selective pressures**

318 Draft versions of the magnetosome gene clusters (MGCs) were reconstructed for the new
319 genomes, and despite the incompleteness of some operons, several observations were
320 validated. First, the *feoABm*, *mms6*, *mamGFDC*, *mamAB* and *mamXY* gene clusters
321 encoding proteins involved in magnetosome biogenesis were found in all genomes with a
322 very high degree of synteny conservation within operons (Fig. 2). Most of the strains carry

323 an additional *mamJOE-like* gene cluster downstream *mamV* and *mamW*. If present, this
324 cluster of paralogs was followed by several orthologous proteins localized upstream *mamXY*
325 operon, among which some are specific to MTB_{Mag}. For instance, an homolog of the ferric
326 uptake regulation protein Fur is often present in the magnetosomes gene cluster and in *M.*
327 *gryphiswaldense* MSR-1, an homolog that was previously shown to affect magnetite
328 biomineralization, is present outside the MGC [78]. With the exception of LM-5, whose
329 MGC organization is almost identical to that of the AMB-1 cluster, strains of the MSR-1
330 group harbour a slightly different structure. The apparent global conservation of the MGC
331 in *Magnetospirillum* spp. could support their orthology in freshwater MTB_{Mag}. However,
332 we found several genomic features, typical of mobile regions and genomic islands that could
333 be responsible for genomic instability, recombination and HGT. Transposases, integrases
334 and recombinases (e.g. homologs to y4qJ, members of the IS110/IS3/IS407 family) were
335 found in the vicinity or within the MGCs of several strains (e.g. AMB-1, ME-1, UT-4, MSR-
336 1), some of which are phage-related or even involved in a functional conjugation system
337 (e.g. *virB6*, *virB8*, *virB9* of type T Type 4 Secretion System) [79, 80]. It should be noted the
338 presence of two copies of *mamJ* in the *mamAB* operon of strain LM-1. Although the
339 percentage of similarity of its encoded protein is low compared to MamJ copies of members
340 of the *Magnetospirillum* genus, it shows that this gene is not restricted to the magnetotactic
341 spirilla from freshwater. In strains MSR-1 and AMB-1, MamJ was shown to interact with
342 MamK filament and thus involved in magnetosome chain formation [81, 82].

343 **Operons involved in magnetotaxis were acquired and lost repeatedly in the course of the** 344 ***Magnetospirillum* evolution**

345 The evolutionary history of magnetotaxis was investigated by reconstructing phylogenies
346 based on known magnetosome-associated proteins described in MSR-1 and AMB-1
347 genomes that are shared by all strains used in this study. We checked the automatic
348 annotation manually gene by gene for rare ambiguous cases. Gene orthology was then
349 tested by comparing gene and species trees topologies. Trees reconstructed from each of the
350 31 *mam* genes/proteins conserved in all *Magnetospirillum* draft and closed genomes were

351 consistent with each other within putative operons. However, some nodes were not always
352 well statistically supported because some amino acids (AA) and DNA sequences were almost
353 identical between genomes. Indeed, for numerous magnetosome-associated proteins, AA
354 sequences identity percentage was up to 25% higher than the average AAI value. For
355 example, MamH sequences were 100% identical between AMB-1, ME-1 and SP-1 while
356 the AAI values of their genomes compared pairwise were between 75 and 87%. Unless a
357 strong negative selection pressure purified polymorphism over *Magnetospirillum* speciation
358 history, such result was congruent with allelic exchange. Trees topologies built from the
359 concatenation of Mam proteins and genes were incongruent with that of the species
360 evolutionary history (Fig. 3). They showed magnetotaxis has a common origin, but does not
361 share the same ancestry in *Magnetospirillum* and has been regularly transferred between
362 ancestors by homologous recombination and/or HGT events. The *mms6/mamGFDC* and
363 *mamAB* operons had the same history (Fig. S7) so data were combined to build one tree
364 (Fig. 3b). This history was different for the *mamXY* operon (including here the *mag* genes)
365 (Fig. 3c). The *feoAB* evolution could not be totally resolved because the polymorphism was
366 too low and the degree of site saturation too high (Fig. 3d). From these trees, the most
367 obvious incongruences between the species tree and magnetotaxis trees were for the
368 relationships between strains LM-5 and CP-1 with the rest of the lineages, even if
369 conflicting signals were globally observed for all strains. Indeed, we confirmed at the whole
370 genome level that although strain LM-5 belongs to the MSR-1 group, its magnetosome
371 genes were most closely related to species of the AMB-1 group [8]. For strain CP-1, isolated
372 in this study, it is the opposite; CP-1 belongs to the AMB-1 group but its MGC shared a
373 more recent ancestry with bacteria of the MSR-1 group.

374 To go further, we inferred the most parsimonious scenarios explaining such
375 evolutionary histories of the magnetosome genes by reconciling these gene trees and the
376 species tree under the duplication-transfer-loss (DTL) model implemented in Notung 2.9
377 software [83] (Fig. 4). Under such a model, the ancestor of the four monophyletic groups of
378 magnetotactic and non-magnetotactic species had a magnetotactic ancestor. Magnetotaxis
379 was then lost in the SpK and MGU-K5 groups (Fig. 4 and Fig. S8). The MGCs of the UT-4

380 and CP-1 strains represent the most ancestral forms of the MGC in the MSR-1 group and
381 AMB-1 group, respectively. However, an ancient HGT event replaced the whole MGC of
382 the most recent MSR-1 group species by an ancestral form that has emerged anciently first
383 in the AMB-1 clade. This event was followed by other similar events of orthologs
384 replacement during the *Magnetospirillum* diversification within and between groups.
385 Importantly, several paralogs of the *mamQ*, *mamR* and *mamB* genes seem to have been
386 horizontally transferred together with the *mamAB* operon during the AMB-1 clade
387 diversification. These genes are identical or nearly identical in the closest lineages of the
388 AMB-1 clade and have likely emerged recently. Further analyses using a larger dataset will
389 be needed to test the evolutionary relationships of these paralogs with the *mamJOE*-like
390 gene cluster. This later likely emerged from duplication of *mamJOE* in the ancestor of all
391 *Magnetospirillum* spp. of AMB-1 group (Fig. 4). The *mamQRB* paralogs present in AMB-1
392 closest relatives seem to have emerged from a different and more recent duplication event
393 than that linking the *mamMOBQ* paralogs in CP-1, SP-1 and SS-4.

394

395 **Discussion**

396 Determining the evolutionary forces shaping genomic polymorphism allows to identify key
397 features involved in microbial adaptation and to resolve their evolutionary histories. In
398 prokaryotes, genetic parallelism was identified by the detection of repeated emergences of
399 the same *de novo* beneficial mutations [37–39]. Here, we showed that multiple allelic
400 exchanges of whole operons mediated the parallel evolution of magnetotaxis in two
401 evolutionarily distinct groups of bacteria. We thus provide evidence that another force than
402 mutation can participate in the maintenance of highly similar phenotypes within a group
403 of phylogenetically distant prokaryotes sharing the same ecological niche. Parallel
404 evolution of distantly related populations is favoured when they experience similar
405 selection pressures [37, 43]. Freshwater magnetotactic species cluster into two genomic
406 genera for which it was difficult, even impossible, to identify biological and ecological
407 boundaries. Although, these two species clades represented by AMB-1 and MSR-1 strains,
408 could represent two different genera and carry on different names, we thus believe that the
409 absence of these boundaries impedes the amending of the *Magnetospirillum* genus name of
410 either the AMB-1 or MSR-1 groups.

411 Here, these results raise several questions about the role of magnetotaxis with
412 microbial adaptation to habitats at the oxic-anoxic interfaces of freshwater sediments. A
413 part of MTB_{Mag} similarity could not be associated to phylogenetic inertia solely, but more
414 likely to adaptation of similar environmental constraints [84]. Did magnetotaxis trigger the
415 ecological cohesiveness of these divergent lineages? Indeed, magnetotaxis is known to
416 optimize bacterial motility along with redox and chemical gradients [2, 85]. Repeated
417 acquisition of beneficial alleles could thus speed up microbial adaptation to microoxic
418 settings by optimizing the sensing or facilitating magnetosome formation in specific
419 conditions. Instead, it is also possible that these narrow and unstable niches selected few
420 specialized catabolic reactions on which magnetosome biogenesis depends on [86–89]. In
421 that case, magnetotaxis selection would arise secondarily from divergent populations
422 already ecologically cohesive that exploit the same redox reactions and carbon sources.
423 According to the theory, this cohesiveness arises when few adaptive solutions exist to face

424 the environment, which in turn fosters the genetic parallelism or even convergence [37].
425 Gene flow and allelic exchange are known to allow the recipient bacteria with pre-existing
426 adaptations to invade a new niche. However, in this specific situation, it can also improve
427 its performance in its current niche [90]. Given our data, this second scenario is the most
428 parsimonious explanation, but in any case, they both support that magnetotaxis is an
429 important adaptation to oxic-anoxic interfaces.

430 For years, studies compiled evidences of the MGC transferability between MTB and
431 non-MTB. Adaptive transfer of genes is limited to those that can be transferred as a
432 functional unit, provide a niche-transcending adaptation, and are compatible with the
433 architecture and physiology of other organisms [42]. To make the exchange possible, genes
434 must fit on a chromosomal segment short enough to be transferred or they must fit on a
435 mobile extrachromosomal element [41]. The MGC totally fits with all these requirements.
436 First, the heterologous expression of *mam* operons in foreign species with compatible
437 physiology led to the formation of magnetosomes [91]. Then, these genes are surrounded
438 by mobile elements from type IV secretion systems involved in conjugation, transposases
439 with a high degree of conservation and phage elements [29, 92]. Interestingly, numerous
440 similar mobile elements are surrounding the magnetosome islet, observed so far in the
441 genome of *M. Magneticum* AMB-1 only [93, 94]. This genomic region harbours few *mam*
442 genes homologs, whose acquisition has been related to an HGT event from the *Ca.*
443 *Etaproteobacteria* strain MC-1. However, this scenario is unlikely because of their
444 homology with *Magnetospirillum mam* genes. Data rather support that the AMB-1 islet is
445 the remnant of one or several independent ancient events (including duplication and/or
446 HGT) that occurred in the early time of *Magnetospirillum* groups emergence. Currently,
447 the lack of sequence conservation and the dramatic evolutionary acceleration of Mam
448 homologs in the islet could result from a neo-functionalization and prevent to fully resolve
449 their history in the light of the current data.

450 So far, the contribution of gene flow and allelic exchange to magnetotaxis evolution
451 was formally evidenced for ancestors of closely related bacterial genera only [34]. The
452 current evolutionary scenario proposes that these forces did not contribute to magnetotaxis

453 evolution at larger taxonomic scales [30, 31]. However, this unexpected scenario relies on
454 big assumptions, which led to other authors to question it and to state that HGT contributed
455 in the early steps of phyla emergences [33]. Only new genomes of deep branching lineages
456 and proper analyses will resolve MTB evolution that very likely involve mutation,
457 recombination and duplication events. The parallel evolution of magnetotaxis observed in
458 the *Magnetospirillum* clades may have occurred at larger taxonomic scales between MTB
459 donors and non-magnetotactic recipient lineages with compatible physiologies and sharing
460 the same ecological niches. The successful transformation of *Rhodospirillum rubrum* [91]
461 with the MSR-1 MGC showed that such event is possible. In most aquatic habitats, oxic-
462 anoxic interface niche represents a tiny layer of ~100 μm in thickness where
463 microaerophilic microorganisms thrive due to the overlap of reduced and oxidized chemical
464 species. This layer is a very coveted zone and thus a hot spot for microorganismal adaptation
465 where adaptive solutions may be reduced [95].

466 Multiple genome sequencing projects revealed years after years the multi-faceted
467 evolution of magnetotaxis with evidences of both vertical and horizontal inheritance [17,
468 31–34]. Despite the polyphyletism of magnetotaxis at the phylum level, some authors
469 argued for a vertical inheritance of the MGC followed by multiple and independent losses
470 in non-MTB clades. Here, we showed further evidence that genetic exchanges may explain
471 this polyphyletism at the genus level.. Because our sample was limited to few strains
472 collected worldwide over a large time scale, we undoubtedly underestimated the number
473 of events, including potential duplications [35]. Repeated *de novo* mutation might have also
474 contributed in some extent to the parallel evolution of the function, but our approach was
475 not suitable to identify such events. In the case of magnetotaxis, recombination between
476 distantly related bacteria could represent a more parsimonious and rapid solution for
477 adaptation compared to independent and repeated *de novo* mutations. The reason comes
478 from the magnetosome gene clusters architecture: magnetosome biogenesis relies on a set
479 of operons involving tens of interacting proteins that certainly coevolved to optimize the
480 function [5]. Thus, any mutation optimizing the function of a single gene would not
481 necessarily optimize the function itself and could even decrease it if other beneficial

482 mutations do not occur at the same time. Selection acting at the operon level rather than
483 on genes individually, homologous recombination of entire operons could rapidly purge the
484 diversity and spread the beneficial mutations in the population. It is important to further
485 test these hypotheses using *MTB_{Mag}* and experimental evolution approaches. Such study
486 would definitively quantify the relative role of gene flow compared to mutation in speeding
487 up adaptation when this later involves large operons of interacting genes and functions as
488 complex as biomineralization.

489

490

491 **Acknowledgments**

492 This work was supported by projects from the French National Research Agency (ANR
 493 Tremplin-ERC: ANR-16-TERC-0025-01 and ANR PRC: ANR-18-CE31-0003). Genome
 494 sequencing was funded thanks to the Appel à Projets en Génomique Environnementale -
 495 CNRS (DJ/ST/IP/2013/D-112). We are grateful to the INRA MIGALE bioinformatics
 496 platform (<http://migale.jouy.inra.fr>) for providing computational resources. We
 497 acknowledge the Institut de Radioprotection et de Sûreté Nucléaire (IRSN) at CEA
 498 Cadarache for the access of the transmission electron microscope Tecnai G2 BioTWIN.

499

500 **Conflicts of interest**

501 The authors declare no conflict of interest.

502

503 **References**

- 504 1. Bazylinski DA, Frankel RB. Magnetosome formation in prokaryotes. *Nat Rev*
 505 *Microbiol* 2004; **2**: 217–230.
- 506 2. Klumpp S, Lefèvre CT, Bennet M, Faivre D. Swimming with magnets: From
 507 biological organisms to synthetic devices. *Phys Rep* 2019; **789**: 1–54.
- 508 3. Schleifer KH, Schüler D, Spring S, Weizenegger M, Amann R, Ludwig W, et al. The
 509 genus *Magnetospirillum* gen. nov. description of *Magnetospirillum gryphiswaldense* sp. nov.
 510 and transfer of *Aquaspirillum magnetotacticum* to *Magnetospirillum magnetotacticum* comb.
 511 nov. *Syst Appl Microbiol* 1991; **14**: 379–385.
- 512 4. Matsunaga T, Sakaguchi T, Tadakoro F. Magnetite formation by a magnetic bacterium
 513 capable of growing aerobically. *Appl Microbiol Biotechnol* 1991; **35**: 651–655.
- 514 5. Uebe R, Schüler D. Magnetosome biogenesis in magnetotactic bacteria. *Nat Rev*
 515 *Microbiol* 2016; **14**: 621–637.
- 516 6. Grant CR, Wan J, Komeili A. Organelle Formation in Bacteria and Archaea. *Annu Rev*
 517 *Cell Dev Biol* 2018; **34**: 217–238.
- 518 7. Schüler D, Spring S, Bazylinski DA. Improved technique for the isolation of
 519 magnetotactic spirilla from a freshwater sediment and their phylogenetic characterization.
 520 *Syst Appl Microbiol* 1999; **22**: 466–471.
- 521 8. Lefèvre CT, Schmidt ML, Vilorio N, Trubitsyn D, Schüler D, Bazylinski DA. Insight
 522 into the evolution of magnetotaxis in *Magnetospirillum* spp., based on mam gene phylogeny.
 523 *Appl Environ Microbiol* 2012; **78**: 7238–7248.

- 524 9. Wang Y, Lin W, Li J, Zhang T, Li Y, Tian J, et al. Characterizing and optimizing
525 magnetosome production of *Magnetospirillum* sp. XM-1 isolated from Xi'an City Moat,
526 China. *FEMS Microbiol Lett* 2015; **362**.
- 527 10. Dziuba M, Koziaeva V, Grouzdev D, Burganskaya E, Baslerov R, Kolganova T, et al.
528 *Magnetospirillum caucaseum* sp. nov., *Magnetospirillum marisnigri* sp. nov. and
529 *Magnetospirillum moscoviense* sp. nov., freshwater magnetotactic bacteria isolated from three
530 distinct geographical locations in European Russia. *Int J Syst Evol Microbiol* 2016; **66**: 2069–
531 2077.
- 532 11. Ke L, Chen Y, Liu P, Liu S, Wu D, Yuan Y, et al. Characteristics and optimised
533 fermentation of a novel magnetotactic bacterium, *Magnetospirillum* sp. ME-1. *FEMS*
534 *Microbiol Lett* 2018; **365**.
- 535 12. Wang Y, Casaburi G, Lin W, Li Y, Wang F, Pan Y. Genomic evidence of the
536 illumination response mechanism and evolutionary history of magnetotactic bacteria within
537 the Rhodospirillaceae family. *BMC Genomics* 2019; **20**: 407.
- 538 13. Lefèvre CT, Bazylinski DA. Ecology, diversity, and evolution of magnetotactic
539 bacteria. *Microbiol Mol Biol Rev* 2013; **77**: 497–526.
- 540 14. Gonçalves RP, Bernadac A, Sturgis JN, Scheuring S. Architecture of the native
541 photosynthetic apparatus of *Phaeospirillum molischianum*. *J Struct Biol* 2005; **152**: 221–228.
- 542 15. Thrash JC, Ahmadi S, Torok T, Coates JD. *Magnetospirillum bellicus* sp. nov., a
543 novel dissimilatory perchlorate-reducing alphaproteobacterium isolated from a bioelectrical
544 reactor. *Appl Environ Microbiol* 2010; **76**: 4730–4737.
- 545 16. Gorlenko VM, Dziuba MV, Maleeva AN, Panteleeva AN, Kolganova TV, Kuznetsov
546 BB. *Magnetospirillum aberrantis* sp. nov., a new freshwater bacterium with magnetic
547 inclusions. *Mikrobiologiya* 2011; **80**: 679–690.
- 548 17. Lin W, Zhang W, Zhao X, Roberts AP, Paterson GA, Bazylinski DA, et al. Genomic
549 expansion of magnetotactic bacteria reveals an early common origin of magnetotaxis with
550 lineage-specific evolution. *ISME J* 2018; 1.
- 551 18. Burgess JG, Kawaguchi R, Sakaguchi T, Thornhill RH, Matsunaga T. Evolutionary
552 relationships among *Magnetospirillum* strains inferred from phylogenetic analysis of 16S
553 rDNA sequences. *J Bacteriol* 1993; **175**: 6689–6694.
- 554 19. Bazylinski DA, Dean AJ, Williams TJ, Long LK, Middleton SL, Dubbels BL.
555 Chemolithoautotrophy in the marine, magnetotactic bacterial strains MV-1 and MV-2. *Arch*
556 *Microbiol* 2004; **182**: 373–387.
- 557 20. Bazylinski DA, Blakemore RP. Nitrogen fixation (acetylene-reduction) in
558 *Aquaspirillum-magnetotacticum*. *Curr Microbiol* 1983; **9**: 305–308.
- 559 21. Bazylinski DA, Dean AJ, Schüler D, Phillips EJ, Lovley DR. N₂-dependent growth
560 and nitrogenase activity in the metal-metabolizing bacteria, *Geobacter* and *Magnetospirillum*
561 species. *Environ Microbiol* 2000; **2**: 266–273.
- 562 22. Bazylinski DA, Blakemore RP. Denitrification and assimilatory nitrate reduction in
563 *Aquaspirillum magnetotacticum*. *Appl Environ Microbiol* 1983; **46**: 1118–1124.
- 564 23. Blakemore RP, Short KA, Bazylinski DA, Rosenblatt C, Frankel RB. Microaerobic
565 conditions are required for magnetite formation within *Aquaspirillum magnetotacticum*.
566 *Geomicrobiol J* 1985; **4**: 53–71.
- 567 24. Schüler D, Baeuerlein E. Dynamics of iron uptake and Fe₃O₄ biomineralization
568 during aerobic and microaerobic growth of *Magnetospirillum gryphiswaldense*. *J Bacteriol*
569 1998; **180**: 159–162.
- 570 25. Heyen U, Schüler D. Growth and magnetosome formation by microaerophilic
571 *Magnetospirillum* strains in an oxygen-controlled fermentor. *Appl Microbiol Biotechnol*
572 2003; **61**: 536–544.

- 573 26. Geelhoed JS, Kleerebezem R, Sorokin DY, Stams AJM, van Loosdrecht MCM.
 574 Reduced inorganic sulfur oxidation supports autotrophic and mixotrophic growth of
 575 *Magnetospirillum* strain J10 and *Magnetospirillum gryphiswaldense*. *Environ Microbiol*
 576 2010; **12**: 1031–1040.
- 577 27. Grünberg K, Wawer C, Tebo BM, Schüler D. A large gene cluster encoding several
 578 magnetosome proteins is conserved in different species of magnetotactic bacteria. *Appl*
 579 *Environ Microbiol* 2001; **67**: 4573–4582.
- 580 28. Grünberg K, Müller E-C, Otto A, Reszka R, Linder D, Kube M, et al. Biochemical
 581 and proteomic analysis of the magnetosome membrane in *Magnetospirillum gryphiswaldense*.
 582 *Appl Environ Microbiol* 2004; **70**: 1040–1050.
- 583 29. Ullrich S, Kube M, Schübbe S, Reinhardt R, Schüler D. A hypervariable 130-kilobase
 584 genomic region of *Magnetospirillum gryphiswaldense* comprises a magnetosome island
 585 which undergoes frequent rearrangements during stationary growth. *J Bacteriol* 2005; **187**:
 586 7176–7184.
- 587 30. Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, Almeida LGP, Vasconcelos ATR, et al.
 588 Monophyletic origin of magnetotaxis and the first magnetosomes. *Environ Microbiol* 2013;
 589 **15**: 2267–2274.
- 590 31. Lin W, Paterson GA, Zhu Q, Wang Y, Kopylova E, Li Y, et al. Origin of microbial
 591 biomineralization and magnetotaxis during the Archean. *Proc Natl Acad Sci U S A* 2017; **114**:
 592 2171–2176.
- 593 32. Jogler C, Kube M, Schübbe S, Ullrich S, Teeling H, Bazylynski DA, et al.
 594 Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides
 595 further evidence for horizontal gene transfer. *Environ Microbiol* 2009; **11**: 1267–1277.
- 596 33. Wang S, Chen Y. Origin of magnetotaxis: Vertical inheritance or horizontal transfer?
 597 *Proc Natl Acad Sci* 2017; **114**: E5016–E5018.
- 598 34. Monteil CL, Perrière G, Menguy N, Ginet N, Alonso B, Waisbord N, et al. Genomic
 599 study of a novel magnetotactic Alphaproteobacteria uncovers the multiple ancestry of
 600 magnetotaxis. *Environ Microbiol* 2018; **20**: 4415–4430.
- 601 35. Du H, Zhang W, Zhang W, Zhang W, Pan H, Pan Y, et al. Magnetosome Gene
 602 Duplication as an Important Driver in the Evolution of Magnetotaxis in the
 603 Alphaproteobacteria. *mSystems* 2019; **4**.
- 604 36. Rundle HD, Nosil P. Ecological speciation. *Ecol Lett* 2005; **8**: 336–352.
- 605 37. Conte GL, Arnegard ME, Peichel CL, Schluter D. The probability of genetic
 606 parallelism and convergence in natural populations. *Proc R Soc B Biol Sci* 2012; **279**: 5039–
 607 5047.
- 608 38. Orr HA. The Probability of Parallel Evolution. *Evolution* 2005; **59**: 216–220.
- 609 39. Gompel N, Prud'homme B. The causes of repeated genetic evolution. *Dev Biol* 2009;
 610 **332**: 36–47.
- 611 40. Maynard Smith J, Smith NH, O'Rourke M, Spratt BG. How clonal are bacteria? *Proc*
 612 *Natl Acad Sci U S A* 1993; **90**: 4384–4388.
- 613 41. Lawrence JG, Retchless AC. The Interplay of Homologous Recombination and
 614 Horizontal Gene Transfer in Bacterial Speciation. In: Gogarten MB, Gogarten JP,
 615 Olendzenski LC (eds). *Horizontal Gene Transfer: Genomes in Flux*. 2009. Humana Press,
 616 Totowa, NJ, pp 29–53.
- 617 42. Wiedenbeck J, Cohan FM. Origins of bacterial diversity through horizontal genetic
 618 transfer and adaptation to new ecological niches. *FEMS Microbiol Rev* 2011; **35**: 957–976.
- 619 43. Bailey SF, Blanquart F, Bataillon T, Kassen R. What drives parallel evolution?: How
 620 population size and mutational variation contribute to repeated evolution. *BioEssays News*
 621 *Rev Mol Cell Dev Biol* 2017; **39**: 1–9.

- 622 44. Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP. The Bacterial Species Challenge:
623 Making Sense of Genetic and Ecological Diversity. *Science* 2009; **323**: 741–746.
- 624 45. Barrick JE, Lenski RE. Genome dynamics during experimental evolution. *Nat Rev*
625 *Genet* 2013; **14**: 827–839.
- 626 46. Cohan FM. Bacterial Species and Speciation. *Syst Biol* 2001; **50**: 513–524.
- 627 47. Nakamura Y, Itoh T, Matsuda H, Gojobori T. Biased biological functions of
628 horizontally transferred genes in prokaryotic genomes. *Nat Genet* 2004; **36**: 760–766.
- 629 48. Vallenet D, Calteau A, Cruveiller S, Gachet M, Lajus A, Josso A, et al. MicroScope in
630 2017: an expanding and evolving integrated resource for community expertise of microbial
631 genomes. *Nucleic Acids Res* 2017; **45**: D517–D528.
- 632 49. Matsunaga T, Okamura Y, Fukuda Y, Wahyudi AT, Murase Y, Takeyama H.
633 Complete genome sequence of the facultative anaerobic magnetotactic bacterium
634 *Magnetospirillum* sp. strain AMB-1. *DNA Res* 2005; **12**: 157–166.
- 635 50. Wang X, Wang Q, Zhang W, Wang Y, Li L, Wen T, et al. Complete Genome
636 Sequence of *Magnetospirillum gryphiswaldense* MSR-1. *Genome Announc* 2014; **2**.
- 637 51. Smalley MD, Marinov GK, Bertani LE, DeSalvo G. Genome Sequence of
638 *Magnetospirillum magnetotacticum* Strain MS-1. *Genome Announc* 2015; **3**.
- 639 52. Grouzdev DS, Dziuba MV, Sukhacheva MS, Mardanov AV, Beletskiy AV, Kuznetsov
640 BB, et al. Draft Genome Sequence of *Magnetospirillum* sp. Strain SO-1, a Freshwater
641 Magnetotactic Bacterium Isolated from the Ol'khovka River, Russia. *Genome Announc* 2014;
642 **2**.
- 643 53. Dzyuba MV, Mardanov AV, Beletskii AV, Kolganova TV, Sukhacheva MV,
644 Shelenkov AA, et al. Reconstruction of iron metabolism pathways of bacteria
645 *Magnetospirillum aberrantis* SpK spp. based on sequenced genome analysis. *Dokl Biol Sci*
646 2012; **444**: 202–205.
- 647 54. Melnyk RA, Engelbrekton A, Clark IC, Carlson HK, Byrne-Bailey K, Coates JD.
648 Identification of a perchlorate reduction genomic island with novel regulatory and metabolic
649 genes. *Appl Environ Microbiol* 2011; **77**: 7401–7404.
- 650 55. Wang Y, Zhang T, Lin W, Zhang B, Cai Y, Yang C, et al. Complete Genome
651 Sequence of *Magnetospirillum* sp. Strain XM-1, Isolated from the Xi'an City Moat, China.
652 *Genome Announc* 2016; **4**.
- 653 56. Ke L, Liu P, Liu S, Gao M. Complete Genome Sequence of *Magnetospirillum* sp. ME-
654 1, a Novel Magnetotactic Bacterium Isolated from East Lake, Wuhan, China. *Genome*
655 *Announc* 2017; **5**.
- 656 57. Ivanovsky RN, Keppen OI, Lebedeva NN, Beletsky AV, Mardanov AV, Grouzdev
657 DS. Draft Genome Sequence of the Anoxygenic Phototrophic Bacterium *Phaeospirillum*
658 *fulvum* MGU-K5. *Genome Announc* 2017; **5**.
- 659 58. Duquesne K, Prima V, Ji B, Rouy Z, Médigue C, Talla E, et al. Draft genome
660 sequence of the purple photosynthetic bacterium *Phaeospirillum molischianum* DSM120, a
661 particularly versatile bacterium. *J Bacteriol* 2012; **194**: 3559–3560.
- 662 59. Contreras-Moreira B, Vinuesa P. GET_HOMOLOGUES, a Versatile Software
663 Package for Scalable and Robust Microbial Pangenome Analysis. *Appl Environ Microbiol*
664 2013; **79**: 7696–7701.
- 665 60. Rodriguez-R LM, Konstantinidis KT. The enveomics collection: a toolbox for
666 specialized analyses of microbial genomes and metagenomes. 2016. PeerJ Inc.
- 667 61. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species
668 delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*
669 2013; **14**: 60.

- 670 62. Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, et al. A Proposed
671 Genus Boundary for the Prokaryotes Based on Genomic Insights. *J Bacteriol* 2014; **196**:
672 2210–2215.
- 673 63. Pantiukh K, Grouzdev D. POCP-matrix calculation for a number of genomes. 2017.
- 674 64. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, et al. MicroScope--
675 an integrated microbial resource for the curation and comparative analysis of genomic and
676 metabolic data. *Nucleic Acids Res* 2013; **41**: D636-647.
- 677 65. Karp PD, Latendresse M, Caspi R. The Pathway Tools Pathway Prediction Algorithm.
678 *Stand Genomic Sci* 2011; **5**: 424–429.
- 679 66. Caspi R, Altman T, Dreher K, Fulcher CA, Subhraveti P, Keseler IM, et al. The
680 MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of
681 pathway/genome databases. *Nucleic Acids Res* 2012; **40**: D742-753.
- 682 67. Abby SS, Néron B, Ménager H, Touchon M, Rocha EPC. MacSyFinder: a program to
683 mine genomes for molecular systems with an application to CRISPR-Cas systems. *PloS One*
684 2014; **9**: e110726.
- 685 68. Abby SS, Rocha EPC. Identification of Protein Secretion Systems in Bacterial
686 Genomes Using MacSyFinder. *Methods Mol Biol Clifton NJ* 2017; **1615**: 1–21.
- 687 69. R Core Team. R: A language and environment for statistical computing. R Foundation
688 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. *R Foundation*
689 *for Statistical Computing, Vienna, Austria*. <https://www.R-project.org/>. .
- 690 70. Dray S, Dufour A-B. The ade4 Package: Implementing the Duality Diagram for
691 Ecologists. *J Stat Softw* 2007; **22**: 1–20.
- 692 71. de Vienne DM, Ollier S, Aguileta G. Phylo-MCOA: a fast and efficient method to
693 detect outlier genes and species in phylogenomics using multiple co-inertia analysis. *Mol Biol*
694 *Evol* 2012; **29**: 1587–1598.
- 695 72. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7:
696 improvements in performance and usability. *Mol Biol Evol* 2013; **30**: 772–780.
- 697 73. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new
698 software for selection of phylogenetic informative regions from multiple sequence
699 alignments. *BMC Evol Biol* 2010; **10**: 210.
- 700 74. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective
701 stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;
702 **32**: 268–274.
- 703 75. Kalyanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS.
704 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017;
705 **14**: 587–589.
- 706 76. Stolzer M, Lai H, Xu M, Sathaye D, Vernet B, Durand D. Inferring duplications,
707 losses, transfers and incomplete lineage sorting with nonbinary species trees. *Bioinformatics*
708 2012; **28**: i409–i415.
- 709 77. Koziyeva VV, Dziuba MV, Ivanov TM, Kuznetsov BB, Skryabin KG, Grouzdev DS.
710 Draft Genome Sequences of Two Magnetotactic Bacteria, *Magnetospirillum moscoviense*
711 BB-1 and *Magnetospirillum marisnigri* SP-1. *Genome Announc* 2016; **4**.
- 712 78. Uebe R, Voigt B, Schweder T, Albrecht D, Katzmann E, Lang C, et al. Deletion of a
713 fur-like gene affects iron homeostasis and magnetosome formation in *Magnetospirillum*
714 *gryphiswaldense*. *J Bacteriol* 2010; **192**: 4192–4204.
- 715 79. Guglielmini J, Néron B, Abby SS, Garcillán-Barcia MP, de la Cruz F, Rocha EPC.
716 Key components of the eight classes of type IV secretion systems involved in bacterial
717 conjugation or protein secretion. *Nucleic Acids Res* 2014; **42**: 5715–5727.
- 718 80. Cury J, Touchon M, Rocha EPC. Integrative and conjugative elements and their hosts:
719 composition, distribution and organization. *Nucleic Acids Res* 2017; **45**: 8943–8956.

- 720 81. Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D. An acidic
721 protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature*
722 2006; **440**: 110–114.
- 723 82. Murat D, Quinlan A, Vali H, Komeili A. Comprehensive genetic dissection of the
724 magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle. *Proc*
725 *Natl Acad Sci U S A* 2010; **107**: 5593–5598.
- 726 83. Chen K, Durand D, Farach-Colton M. NOTUNG: a program for dating gene
727 duplications and optimizing gene family trees. *J Comput Biol J Comput Mol Cell Biol* 2000;
728 **7**: 429–447.
- 729 84. Boratyński Z, Brito JC, Campos JC, Cunha JL, Granjon L, Mappes T, et al. Repeated
730 evolution of camouflage in speciose desert rodents. *Sci Rep* 2017; **7**: 3522.
- 731 85. Bennet M, McCarthy A, Fix D, Edwards MR, Repp F, Vach P, et al. Influence of
732 Magnetic Fields on Magneto-Aerotaxis. *PLoS ONE* 2014; **9**: e101150.
- 733 86. Wang K, Ge X, Bo T, Chen Q, Chen G, Liu W. Interruption of the denitrification
734 pathway influences cell growth and magnetosome formation in *Magnetospirillum*
735 *magneticum* AMB-1. *Lett Appl Microbiol* 2011; **53**: 55–62.
- 736 87. Li Y, Katzmann E, Borg S, Schüler D. The periplasmic nitrate reductase Nap is
737 required for anaerobic growth and involved in redox control of magnetite biomineralization in
738 *Magnetospirillum gryphiswaldense*. *J Bacteriol* 2012; **194**: 4847–4856.
- 739 88. Li Y, Raschdorf O, Silva KT, Schüler D. The terminal oxidase cbb3 functions in redox
740 control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. *J Bacteriol*
741 2014; **196**: 2552–2562.
- 742 89. Li Y, Sabaty M, Borg S, Silva KT, Pignol D, Schüler D. The oxygen sensor MgFnr
743 controls magnetite biomineralization by regulation of denitrification in *Magnetospirillum*
744 *gryphiswaldense*. *BMC Microbiol* 2014; **14**: 153.
- 745 90. Cohan FM, Koeppl AF. The origins of ecological diversity in prokaryotes. *Curr Biol*
746 *CB* 2008; **18**: R1024-1034.
- 747 91. Kolinko I, Lohße A, Borg S, Raschdorf O, Jogler C, Tu Q, et al. Biosynthesis of
748 magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene
749 clusters. *Nat Nanotechnol* 2014; **9**: 193–197.
- 750 92. Schübbe S, Kube M, Scheffel A, Wawer C, Heyen U, Meyerdierks A, et al.
751 Characterization of a spontaneous nonmagnetic mutant of *Magnetospirillum gryphiswaldense*
752 reveals a large deletion comprising a putative magnetosome island. *J Bacteriol* 2003; **185**:
753 5779–5790.
- 754 93. Rioux J-B, Philippe N, Pereira S, Pignol D, Wu L-F, Ginet N. A second actin-like
755 MamK protein in *Magnetospirillum magneticum* AMB-1 encoded outside the genomic
756 magnetosome island. *PloS One* 2010; **5**: e9151.
- 757 94. Abreu N, Mannoubi S, Ozyamak E, Pignol D, Ginet N, Komeili A. Interplay between
758 two bacterial actin homologs, MamK and MamK-Like, is required for the alignment of
759 magnetosome organelles in *Magnetospirillum magneticum* AMB-1. *J Bacteriol* 2014; **196**:
760 3111–3121.
- 761 95. Brune A, Frenzel P, Cypionka H. Life at the oxic-anoxic interface: microbial activities
762 and adaptations. *FEMS Microbiol Rev* 2000; **24**: 691–710.

763

764

765 **Figure legends**

766 **Fig. 1** Genomic insight into the evolution and similarities of *Magnetospirillum* relatives. **a**
 767 Phylogeny of *Magnetospirillum* species rooted with strains representing other
 768 Rhodospirillaceae species (grey). The Maximum-likelihood tree was drawn to scale and
 769 branches length represents the number of base substitutions per site. Black circles represent
 770 nodes supported by 100% of the replicates. Names in bold represent MTB strains. Strains
 771 related by branches with the same colour represented different clades distinguished here,
 772 and could be considered as different genera according to the average amino-acid identity
 773 (AAI) (Fig. S2) and their lifestyle. **b** Histograms drawn to scale showing the number of
 774 orthologous proteins specific to a group of bacteria that are shared by all members of this
 775 group (black bars represent 100 proteins). The first, second and third histograms give the
 776 number of proteins specific to (1) each clade, (2) the MSR-1/SpK group vs. AMB-
 777 1/*Phaeospirillum* group, and (3) MTB groups vs. non-MTB, respectively. **c** Pairwise
 778 comparison of percentages of conserved proteins (POCP) (Fig. S3) and average amino-acid
 779 identities (AAI) between the strains of the four groups ordered according to the
 780 phylogenetic tree presented in panel **a**. Circles and colours are proportional to the values
 781 that represent averages and standard errors calculated from the pairwise comparisons
 782 within each group. All the values are significantly different from each other (Student t-test,
 783 $n=23$, $P < 0.05$). **d** Principal component analysis of the metabolic networks of strains
 784 predicted with the MicroCyc resource implemented in MicroScope with the projection of
 785 ellipses and gravity centres of classes representing the phylogenetic groups.

786 **Fig. 2** Composition and gene synteny of the *feoABm*, *mms6*, *mamGFDC*, *mamAB* and
 787 *mamXY* operons encoding for proteins involved in magnetosome biogenesis in
 788 magnetotactic *Magnetospirillum* species and other conserved genes and *mam* paralogs with
 789 putative function in magnetosome biogenesis. Sequences are organized according to species
 790 phylogeny. Homologous genes are symbolized by the same letters and their relative 5'-3'
 791 orientation compared to each other's is given by the bottom line colour (red or black). A
 792 vertical blue dotted line between two genes symbolizes a truncation (contig edge) while a

793 grey slash denotes that two genes are related through a continuous genomic region of one
 794 or more genes. Genes are next to each other if they are not separated by a slash. The
 795 additional paralogous *mam* genes (the *mamJOE-like* operon or a duplicated genes set of
 796 *mms6*, *mmsF*, *mag1* and *mag3* for example) are associated with a quotation mark.
 797 Paralogous *mam* genes were found on very short contigs outside the MGC in CP-1 and were
 798 not shown on this figure (*mamOBQ* and *feoABm* for example). Genes *h1* to *h4*, *m1* to *m3*
 799 and *fur_m* represent unknown homologous genes conserved in many MGCs, the *mag* genes
 800 previously described [34] and an homolog of the ferric uptake regulator *fur* specific to the
 801 MGC, respectively. The accession number of these genes in the reference genome AMB-1
 802 are BAE49759, BAE49764, BAE49812, BAE49814, BAE49823, BAE49824, BAE49825 and
 803 BAE4981, respectively.

804 **Fig. 3** Evolutionary history of proteins involved in magnetotaxis in *Magnetospirillum*
 805 relatives. **a** Species tree from Figure 1a. Gene trees built from the concatenation of Mam
 806 proteins present in **b** *mms6*, *mamGFDC* and *mamAB* operons, **c** the *mamXY* operon
 807 (including here the *mag1*, *2* and *3* genes) and **d** the *feoABm* operon. Strains names and
 808 external branches are coloured according to the sub-groups of the first panel to facilitate
 809 the visualisation of relationships in gene trees. Gene trees were rooted with the other
 810 magnetotactic Alphaproteobacteria of the Figure 1a including strain LM-1.

811 **Fig. 4** Most parsimonious scenario describing the evolutionary history of *mms6*, *mamGFDC*
 812 and *mamAB* operons along with the diversification of *Magnetospirillum* species. Events of
 813 gene duplication (D), loss (L) and transfer (T) were inferred using the duplication-transfer-
 814 loss (DTL) model of Notung 2.9 software [76] by reconciling the tree based on the
 815 concatenation of shared *mam* AA sequences (thin cladogram, Fig. 2b) with the species tree
 816 (thick grey cladogram, Fig. 1). Colours symbolize divergent clades or lineages of the species
 817 tree. The colour of the internal branches show the ancestry of the genes encoding for
 818 magnetosome-associated proteins. For horizontal gene transfer events, the name of the
 819 donor lineage associated is given. The black solid lines represent the vertical inheritance of
 820 the genes while the black dotted lines represent their loss. D* denotes a putative duplication

821 event that could have occurred in the ancestor of AMB-1 clade and led to the emergence of
822 both ancestors of the *mamJOE-like* cluster.