

1

2

3

4

5 **Evolutionary sparse learning with paired species contrast reveals the**
6 **shared genetic basis of convergent traits**

7

8

9

10 John B. Allard^{1,2}, Sudip Sharma^{1,2}, Ravi Patel^{1,2}, Maxwell Sanderford^{1,2}, Koichiro Tamura^{3,4},
11 Slobodan Vucetic⁵, Glenn S. Gerhard^{*6}, and Sudhir Kumar^{*1,2,7}

12

13 ¹ Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA 19122,
14 USA

15 ² Department of Biology, Temple University, Philadelphia, PA 19122, USA

16 ³ Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan

17 ⁴ Research Center for Genomics and Bioinformatics, Tokyo Metropolitan University, Tokyo,
18 Japan

19 ⁵ Department of Computer and Information Sciences, Temple University, Philadelphia PA, United
20 States of America

21 ⁶ Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA.

22 ⁷ Center for Excellence in Genome Medicine and Research, King Abdulaziz University, Jeddah,
23 Saudi Arabia

24

25 **Corresponding authors* (s.kumar@temple.edu and gsggerhard@temple.edu)

26

27

28 **Cases abound in which nearly identical traits have appeared in distant species facing**
29 **similar environments. These unmistakable examples of adaptive evolution offer**
30 **opportunities to gain insight into their genetic origins and mechanisms through**
31 **comparative analyses. Here, we present a novel comparative genomics approach to build**
32 **genetic models that underlie the independent origins of convergent traits using**
33 **evolutionary sparse learning. We test the hypothesis that common genes and sites are**
34 **involved in the convergent evolution of two key traits: C4 photosynthesis in grasses and**
35 **echolocation in mammals. Genetic models were highly predictive of independent cases**
36 **of convergent evolution of C4 photosynthesis. These results support the involvement of**
37 **sequence substitutions in many common genetic loci in the evolution of convergent**
38 **traits studied. Genes contributing to genetic models for echolocation were highly**
39 **enriched for functional categories related to hearing, sound perception, and deafness (P**
40 **$< 10^{-6}$); a pattern that has eluded previous efforts applying standard molecular**
41 **evolutionary approaches. We conclude that phylogeny-informed machine learning**
42 **naturally excludes apparent molecular convergences due to shared species history,**
43 **enhances the signal-to-noise ratio for detecting molecular convergence, and empowers**
44 **the discovery of common genetic bases of trait convergences.**

45

46 Organisms continuously adapt to their natural environment. Under similar environmental
47 conditions, the same adaptations may evolve independently in clades across the tree of life. For
48 example, the convergent evolution of the ability to echolocate in some bats and toothed whales
49 is an example of adaptation brought on by major transitions to new environments requiring
50 similar physiological innovations. Evolutionary biologists have long sought the common genetic
51 basis of these convergent adaptations under the hypothesis that the same pathways, genes,
52 and/or base substitutions are involved in these adaptations. However, “*the extent to which*
53 *convergent traits evolve by similar genetic and molecular pathways is not clear*”¹. Despite many
54 molecular evolutionary investigations, the strongest evidence for molecular convergence thus
55 far appears to be a marginally significant (FDR-corrected $P = 0.0486$) enrichment of sound
56 perception genes in which convergent and parallel amino acid substitutions were observed²⁻⁴.
57 Although these results hint at the possible presence of some shared genetic basis in the evolution
58 of echolocation in independent clades, some studies could not detect such an enrichment³,
59 casting doubt on the robustness of the results, the general applicability of the methodology, or
60 even the presence of a common genetic basis.

61 The lack of consistent and statistically significant results may be due to insufficient commonality
62 in the genetic bases of these traits, i.e., different genes and different sites may perform similar
63 functions in independent clades. Alternatively, the lack of sufficient statistical power or inability to
64 fully exclude non-adaptive convergence may be hampering efforts to detect genes and sites
65 associated with the evolution of convergent traits⁵⁻⁷. Furthermore, current state-of-the-art
66 approaches primarily reveal retrospective patterns, but they do not explicitly model quantitative
67 genetic changes in convergent trait evolution to make statistical predictions of the presence or
68 absence of the convergent trait.

69 We have addressed these challenges by building predictive genetic models of convergent trait
70 evolution using evolutionary sparse learning (ESL). ESL is supervised machine learning in
71 which genomic components (e.g., genes and sites) are model parameters, and substitutions in
72 multiple sequence alignments are observations⁸. We developed a paired species contrast (PSC)
73 design to select the training data for machine learning to automatically mask neutral
74 (background) sequence convergence that can lead to spurious inferences and reduce the power
75 to detect the genetic basis of convergence^{5,6,9}. Importantly, ESL-PSC simultaneously considers
76 all genetic loci and their respective substitutions during computational analysis, eliminating
77 biases due to arbitrary evolutionary conservation thresholds and convergent substitution cut-offs
78 necessary in some other approaches^{2,3,7,10,11}.

79 ESL-PSC produces a quantitative genetic model to predict the presence/absence of a
80 convergent trait in any species based on its genome sequence. This is needed to test the
81 biological hypothesis of commonality of genetic basis in the independent evolution of the same
82 trait. Lists of loci comprising the genetic model can be subjected to additional analysis to test if
83 there is an enrichment of functional categories relevant to the trait analyzed^{12,13}. This approach
84 is commonly used to establish the biological relevance of candidate loci derived from
85 large-scale scans for molecular convergence in the absence of alternatives^{2-4,9,14-16}. We applied
86 ESL-PSC to build genetic models of convergent evolution of C4 photosynthesis in grasses and
87 of echolocation in mammals because they have been extensively investigated previously^{4,17-22}.

88 **ESL-PSC for building genetic models of convergent traits**

89 We introduce ESL-PSC with an analysis of protein sequence alignments of chloroplast proteins,
90 which are well-suited for demonstrating the predictive ability of the method in a range of grass
91 species that have acquired C4 photosynthesis independently. One may alternatively use
92 ESL-PSC for nucleotide sequence alignments with the option to group sites into exons, introns,

93 or other types of domains and functional annotations, as described in the *Material and Methods*
94 section.

95 ESL uses logistic regression to infer a genetic model that can predict trait-positive and
96 trait-negative species, which we numerically encode as +1 and -1, respectively^{8,23}. In this
97 analysis, the Least Absolute Shrinkage and Selection Operator (LASSO) compares alternative
98 genetic models by imposing penalties for including additional amino acid positions and genes
99 into the model while seeking high prediction accuracy. ESL-PSC produces models that
100 incorporate only those proteins whose member sites make a significant contribution to the ability
101 of the genetic model to classify species according to their traits rather than their ancestry.

102 To train the ESL model, we use a paired species contrast (PSC) approach in which a balanced
103 training dataset of equal numbers of trait-positive and trait-negative species (those with and
104 without the trait of interest, respectively) is first selected such that for every trait-positive
105 species, we include one closely-related trait-negative species. In PSC, species pairs are
106 required to be from evolutionarily independent clades to avoid introducing evolutionary
107 correlations among pairs due to shared evolutionary history, which is known to cause spurious
108 associations^{5,6,9}. As an example, we could select trait-positive species A_1 and D_1 and
109 trait-negative species B_1 and C , respectively, to satisfy the above conditions (**Fig. 1A**).

110 PSC selection of training data ensures that the most recent common ancestor (MRCA) of each
111 trait-positive and trait-negative species pair selected will be more recent than the MRCA of
112 either member of the pair with any of the other species in the analysis. In the above example,
113 the MRCA of A_1 and B_1 (Y) is more recent than that of A_1 and F (W). Also, ESL-PSC
114 automatically excludes all branches in the phylogeny that are unrelated to the evolution of the
115 convergent trait (dotted branches in **Fig. 1A**). This means that the model learning is directly
116 focused on the molecular evolutionary changes between trait-positive and trait-negative species
117 (solid blue and red branches, respectively). If there are multiple species in some trait-positive
118 and trait-negative clades, different combinations of training sets may be used to build separate
119 genetic models followed by model averaging (see *Material and Methods*). ESL-PSC analysis
120 produces a list of proteins included in the genetic model, the estimated relative importance of
121 each locus, and an equation to predict the presence/absence of the trait in a species based on
122 its genetic sequences. Species not used for training for a given model can be utilized for testing
123 the model.

124

125

126 **A**

127

128

129

130

131

132

133

134

135

136

137

138

139

140

141

142

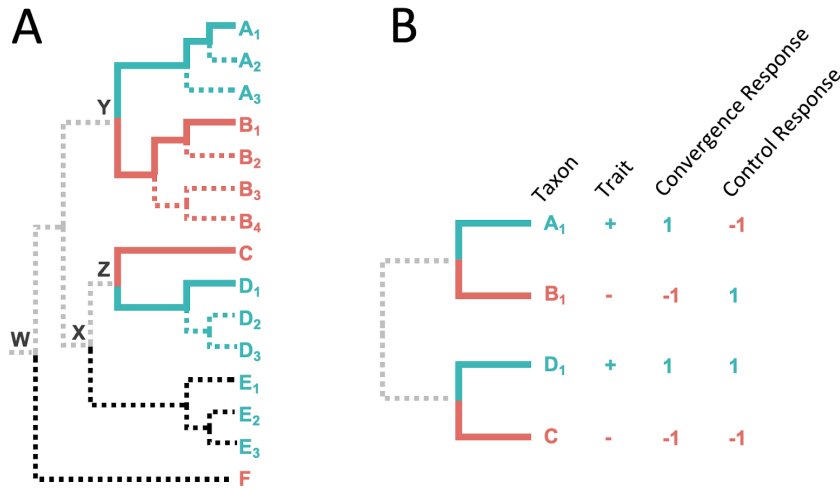


Figure 1. The paired species contrast (PSC) design. **A:** An example phylogeny with one set of selected species (solid blue and red lines). Extraneous lineages (black dotted lines) and shared evolutionary history (gray dotted lines). **B:** A schematic depiction of the four species selected for ESL-PSC analysis. In the ESL experiment, the response variable refers to the binary phenotype, where +1 represents the convergent trait, and -1 represents the ancestral trait.

143 Genetic Models for Convergent Acquisition of C4 Photosynthesis

144 We applied ESL-PSC to build genetic models of photosynthesis evolution using a 64-species
145 alignment of 67 chloroplast proteins²² (see *Material and Methods*). Many of these grass species
146 have convergently evolved the C4 photosynthetic pathway for carbon concentration^{24,25}, while
147 others have retained the ancestral C3 photosynthetic pathway. Previous studies of the genetic
148 basis of C4 evolution have found convergent amino acid substitutions in
149 Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) to be strongly associated with C4
150 evolution, but Casola and Li²² have recently suggested the involvement of other chloroplast
151 proteins as well. However, the extent to which chloroplast proteins other than RuBisCo
152 represent a predictable and common evolutionary basis of C4 evolution remains uncertain.

153 There are six clades in the molecular phylogeny that contain sibling species of both C4 and C3
154 phenotypes (**Fig. 2**), which yielded six pairs of species satisfying the PSC design. Each pair
155 contained a species with C4 photosynthesis and its most closely related species with C3
156 photosynthesis. Because some clades contain multiple candidate trait-positive (C4) and
157 trait-negative (C3) species, we selected the species with the least missing data in the sequence
158 alignment in our first analysis (solid lines in **Fig. 2**). The lengths of individual protein sequence
159 alignments varied from 30 to 1,528 amino acids, with a total of 16,362 positions in 67
160 chloroplast proteins²².

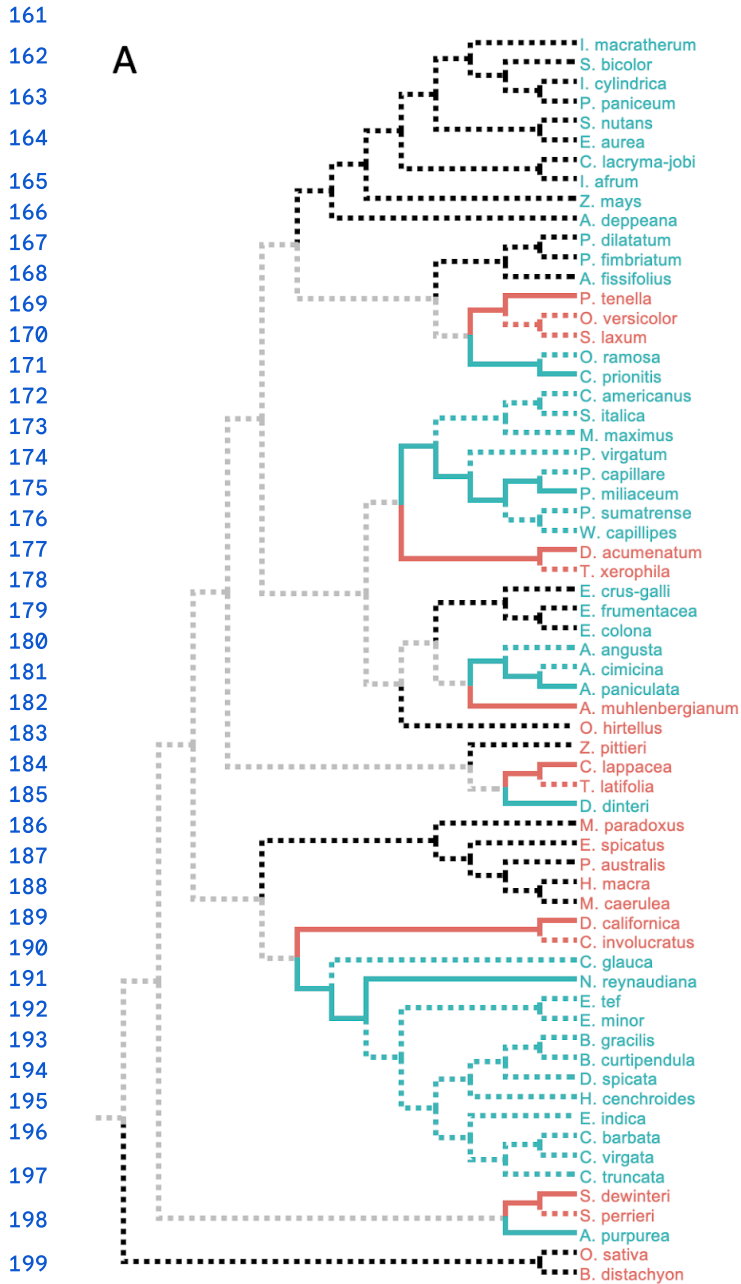


Figure 2. ESL-PSC modeling of convergent acquisition of C4 photosynthesis. A. Experimental design.

An evolutionary tree of 64 grass species based on the phylogeny in Casola and Li²². From the 64 available species, 6 pairs of trait-positive (C4) and trait-negative (C3) species were chosen according to the PSC approach. Where multiple species met the topological requirements for a contrast pair, we selected the two species that were closest in the evolutionary distance and that had the fewest gaps in the alignments. Selected species are shown as solid line branches, and all other branches are depicted as dashed lines. Solid lines begin at the internal node that represents the common ancestor of each pair, and the black (C4) and red (C3) branches represent the unshared ancestry of each selected species. Thus substitutions on these branches can be included in ESL-PSC modeling. Blue (C4) and red (C3) dashed lines represent alternative sibling species of the selected species. Black dashed branches represent clades that are evolutionarily independent of the contrast pairs. These include both C4 and C3 species. Gray branches represent the evolutionary history that is shared equally by selected C4 and C3 species, which we expect to cancel out automatically in the modeling process.

200
201 In ESL-PSC analysis, sparsity penalties must be specified for the inclusion of sites and proteins
202 in the genetic model built using LASSO. These penalties dictate the number of proteins and
203 sites allowed in the genetic model⁸. We used a series of penalties and compared resulting
204 genetic models by using a newly developed Model Fit Score (MFS), which is analogous to the
205 Brier score in logistic regression (see Methods). The genetic model with the best MFS contained
206 included RuBisCo, consistent with previous experimental and analytical knowledge^{20,22,26,27}. This
207 model correctly assigned all six C4 and six C3 species used to train the model and correctly
208 predicted 97% of the other C4 species in this dataset (36 of 37) and 100% of C3 species (15 of

209 15) for a balanced accuracy of 98.5%. An ensemble of genetic models with similar MFS scores
210 (**Fig. 3**) also performed equally well (**Fig. 4A**).

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

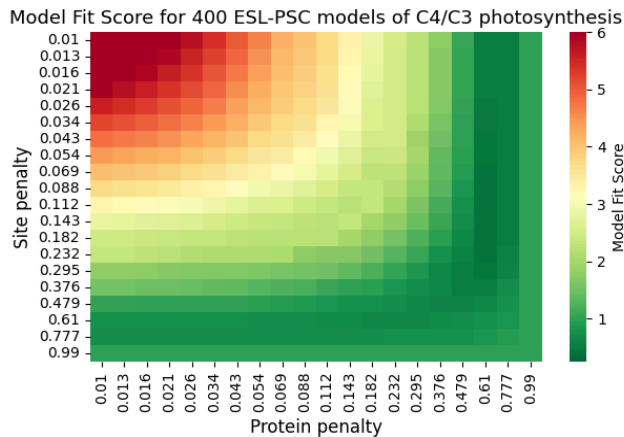
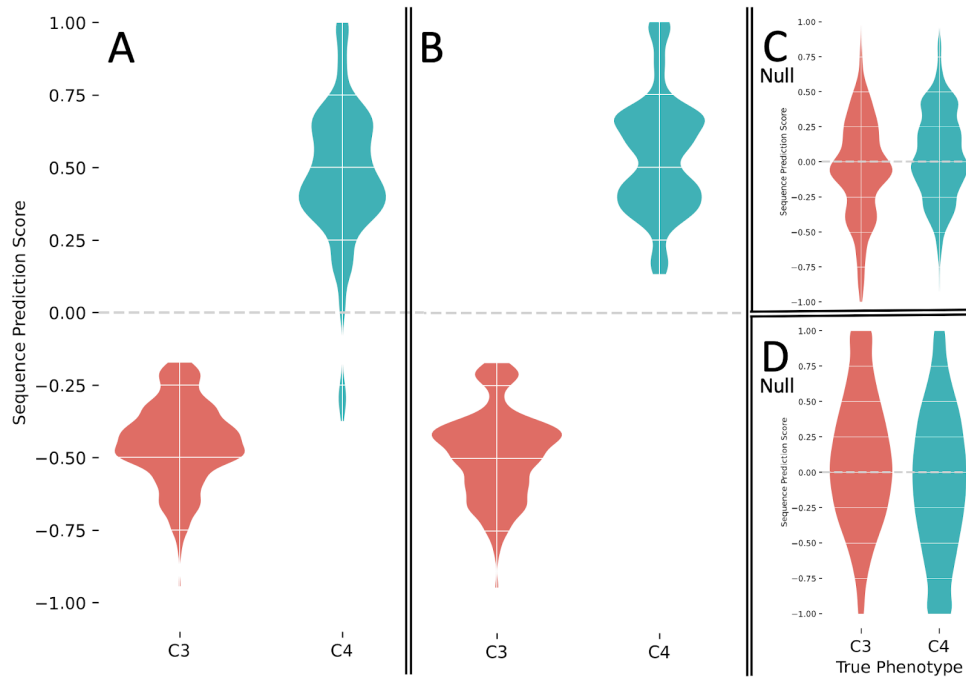


Figure 3 Heat map of Model Fit Scores. 20 values for each inclusion penalty (site and protein) were sampled from a logspace ranging from 1-99% of the maximum non-trivial penalty. A higher MFS suggests a higher risk of overfitting. Models with the best (lowest) 5% of MFS are included in predictive ensembles (Fig. 4, 5).

226 The best MFS model was found to be equally accurate in predicting C4 species that are siblings
227 of those used in the training set, which suggests that multiple C4 species within a clade
228 inherited the trait from a common ancestor. This is consistent with the parsimonious
229 reconstruction of independent C4 trait evolution²⁸. For this reason, genetic models built using
230 different species combinations were also highly accurate (96%, **Fig. 5B**). The best MSF models
231 were also highly predictive of the C4/C3 status of species from independent clades (black
232 dotted branches in **Fig. 2**) that did not contribute any species for training the model (100%
233 accuracy; **Fig. 4B**). This result suggests that many of the same substitutions contributed to C4
234 evolution independently.

235 In addition, we found that evolutionarily-naive machine learning, which did not use the PSC
236 design, could only achieve 64% accuracy in correctly identifying C4 species in the independent
237 clades (black branches in **Fig. 2**). In this experiment, we conducted a direct comparison by
238 selecting 100 input sets of six C4 and six C3 species from among the siblings of the PSC
239 species, but without respecting the PSC design. For these “naive” models, the prediction
240 accuracy fell considerably. In particular, the average true positive rate (TPR), a measure of the
241 ability of the model to recognize C4 species on the basis of information in convergent sites, was
242 only 64% over all of these ensembles compared with 94% for the ensembles built using the
243 PSC approach (**Fig. 4B**). This reduction in accuracy reflects the fact that non-PSC models may
244 incorporate not only sites whose residues are correlated with the phenotype due to convergent
245 evolution but also sites correlated with the phenotype purely due to shared ancestry within the

246 inputs. The latter type of sites carries no information relevant to the prediction of phenotype in
247 clades whose trait-positive species have acquired the trait independently. This result establishes
248 that our PSC design can produce much better genotype-phenotype models than naive machine
249 learning.



250 **Figure 4. Predictive ability of ESL-PSC genetic models of C4/C3 photosynthesis. A-D** Sequence
251 prediction scores (SPS) from model ensembles are shown for known C4 (blue) and C3 (red) species in
252 kernel density estimation plots. Negative SPS indicates a prediction of the C3 phenotype (trait-negative),
253 and positive SPS indicates a prediction of the C4 phenotype. Predictions shown are for all species (A),
254 species in clades independent of the clades contributing species for model training (B). Response-flipped
255 null ESL-PSC models of C4/C3 photosynthesis (C). Null models were constructed by flipping the
256 phenotype response values of 3 out of 6 of the input contrast pairs. This was done for all 10 distinct
257 combinations of 3 out of 6 contrast pairs, and all model predictions were aggregated. SPSs from the best
258 5% of models by MFS are included. Pair-randomized null ESL-PSC models of C4/C3 photosynthesis (D).
259 Null models were constructed by randomly flipping or not flipping the residues between each species
260 contrast pair at every variable residue in the MSA. For each of the 25 alternative PSC input species
261 combinations, randomized pair-flipped alignments were generated, and model ensembles were produced
262 for each. Aggregated predictions are shown.

263

264 Studies of convergence in C4 have focused heavily on RuBisCo, the most abundant enzyme,
265 which has multiple sites of convergent amino acid substitutions in multiple different lineages of
266 plants^{20–22,26,29}. However, we tested the hypothesis that other chloroplast proteins also
267 contributed to C4 evolution by building ESL-PSC models excluding RuBisCo and testing model
268 accuracy in predicting the presence of C4. The RuBisCo-free models had 89% accuracy,

269 suggesting that the convergent basis of the C4 trait extends to other chloroplast genes (**Fig. 5a**).
270 Interestingly, these models correctly predicted C4 photosynthesis in *Alloteropsis angusta*, which
271 was the only false negative for the model containing RuBisCo. *A. angusta* is known to have
272 undergone a C3 to C4 transition independently from the other members of its own genus,
273 including *A. paniculata*³⁰. We found *A. angusta* to be lacking key amino acid substitutions in
274 RuBisCo that are highly diagnostic of other C4 species. Therefore, chloroplast proteins other
275 than RuBisCo have likely contributed significantly to C4 evolution in this case, and more
276 generally. While Casola and Li²² hinted at such a possibility, their statistical analyses using a
277 convergence counting approach did not find a significant excess of convergent substitutions in
278 C4 species as compared to the background C3 species. Therefore, the ESL-PSC framework
279 provided a powerful new way to investigate the genetics of convergent traits and test
280 hypotheses that have not been possible until now.

281

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301 **Convergent Evolution of Echolocation**

302 The independent acquisition of echolocation in bats and whales is among the most well-studied
303 cases of convergent molecular and trait evolution. We selected the microbat *Myotis lucifugus*
304 and the bottlenose dolphin *Tursiops truncatus* as trait-positive species (echolocators) because
305 previous studies involving exome-scale searches for convergence in echolocating mammals
306 have often focused on the comparison of microbats and toothed whales^{2,3,9,31}. In the PSC

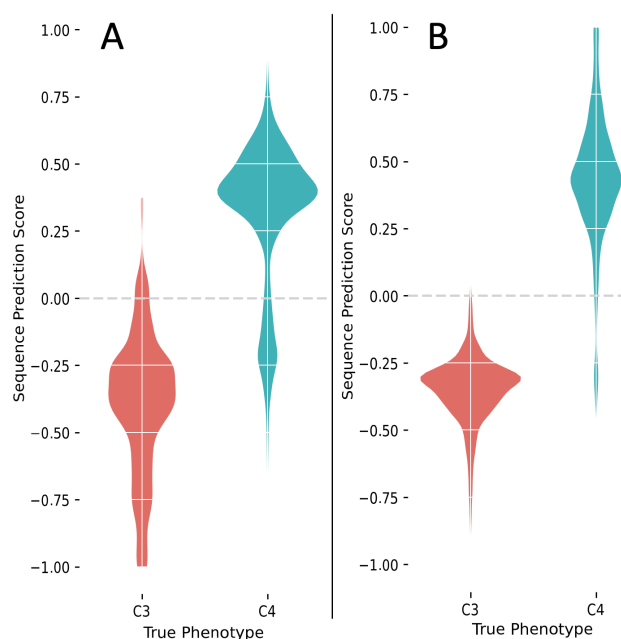
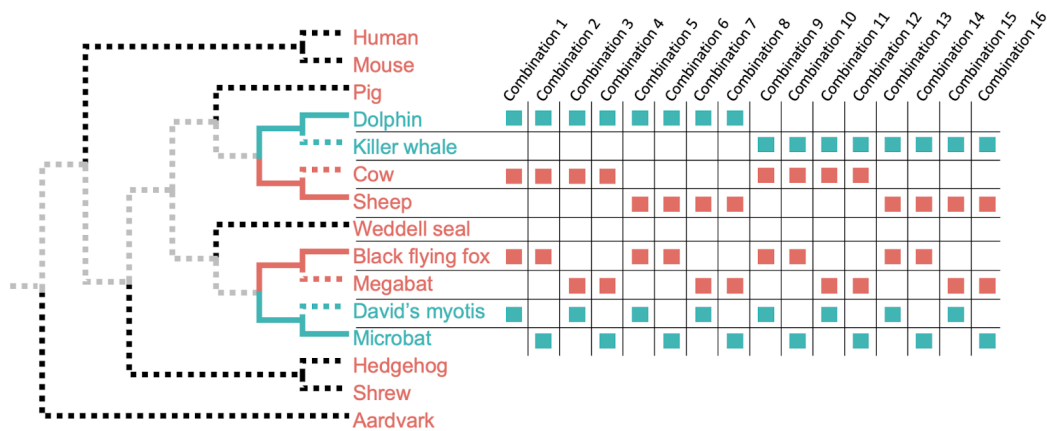


Figure 5. Alternative models. **A:** Predictions from models developed without the inclusion of RuBisCo are shown for independent species. **B:** Alternative PSC combinations. 100 alternative species combinations of PSC pairs were generated, and ensemble models were constructed as above. Predictions were aggregated for only the independent clades (black branches in **Fig. 2**). SPSs from the best 5% of models by MFS are shown from the aggregate of all ensemble models.

307 design, we selected a non-echolocating sister species *Pteropus vampyrus* (megabat) for
308 echolocating *Myotis lucifugus* and non-echolocating *Ovis aries* (sheep) for echolocating
309 bottlenose dolphin *Tursiops truncatus* (**Fig. 6**; see *Methods*). We retrieved 14,509 protein
310 alignments from the OrthoMaM database of orthologous protein-coding sequences for
311 mammalian genomes³².



312 **Figure 6. Echolocation analysis.** Echolocation evolved twice in mammals in our dataset. Therefore two
313 contrast pairs can be constructed (solid blue branches, echolocating; solid red branches,
314 non-echolocating). A series of 15 comparable sets of input pairs can be constructed by using alternative
315 species (dashed blue and red sibling species) in all possible combinations. Species not included in the
316 contrast pairs do not affect the analysis (black dashed branches). Shared ancestry is canceled out (gray
317 branches).

318

319 Because there were only two clades, and thus only two species pairs, we made inferences from
320 a collection of ESL models obtained using a range of sparsity penalties (see *Methods*) and
321 species combinations (**Fig. 6**). The collection of genetic models was then used to generate a
322 ranked list of candidate proteins associated with convergent evolution (**Table S1**). Among the
323 highest-ranked proteins, many were those previously characterized to have signatures of
324 molecular convergence in echolocators, including, Prestin (SLC26a5), TMC1, PJKV (DFNB59),
325 CDH23, CASQ1, and CABP2^{3,17,18,33-35}. In some cases, specific amino acid sites within these
326 proteins have been implicated in conferring the functional changes necessary for the
327 echolocation phenotype, revealed by laboratory assays where mutations to residues found in
328 echolocating species were observed to alter protein function in a manner consistent with
329 echolocation^{3,19}.

330 We generated multiple-tests adjusted *P*-values to gauge the functional enrichment in the
331 top-ranking proteins included in the genetic models. We tested for ~20,000 biological processes

332 and phenotypes (see *Methods*) and found the top 100 proteins to be highly enriched for the
333 “sensory perception of sound” genes (GO:0007605) with an adjusted P -value $< 10^{-4}$ (**Table 1**).
334 This is an improvement in the statistical significance of more than two orders of magnitude
335 compared to the best previous findings of this term (adjusted $P = 0.049$) in FDR-corrected
336 analyses^{4,31}. Our enrichment P -value was highly significant even for 50, 150, and 200 top
337 proteins in the genetic models ($P < 10^{-3}$), suggesting that our results are robust to the size of the
338 gene list analyzed.

339

340 **Table 1. Ontology term enrichments.** Enrichment tests were performed for Gene, Phenotype, and
341 Disease ontology terms for the top 100 highest-ranking trait proteins in our echolocation multiple species
342 combination ensemble model integration analysis. In each figure, the 10 ontology terms with the lowest
343 p -values are shown from each enrichment analysis.

Term	P-value	Adjusted P-value
Go Biological Process		
sensory perception of sound (GO:0007605)	$< 1 \times 10^{-7}$	$< 1 \times 10^{-4}$
sensory perception of mechanical stimulus (GO:0050954)	$< 1 \times 10^{-6}$	$< 1 \times 10^{-3}$
MGI Mammalian Phenotype Level 4		
cochlear inner hair cell degeneration MP:0004398	$< 1 \times 10^{-6}$	$< 1 \times 10^{-3}$
cochlear ganglion degeneration MP:0002857	$< 1 \times 10^{-6}$	$< 1 \times 10^{-3}$
head tossing MP:0005307	$< 1 \times 10^{-6}$	$< 1 \times 10^{-3}$
increased or absent threshold for auditory brainstem response MP:0011967	$< 1 \times 10^{-6}$	$< 1 \times 10^{-3}$
organ of Corti degeneration MP:0000043	$< 1 \times 10^{-5}$	$< 1 \times 10^{-3}$
deafness MP:0001967	$< 1 \times 10^{-4}$	$< 1 \times 10^{-2}$
DisGeNet		
Sensorineural hearing loss, bilateral	$< 1 \times 10^{-8}$	$< 1 \times 10^{-5}$
Nonsyndromic Deafness	$< 1 \times 10^{-4}$	$< 1 \times 10^{-1}$

344

345 This top-100 gene list was also significantly enriched (adjusted $P < 1 \times 10^{-6}$) for many Phenotype
346 Ontology (PO) terms directly related to hearing and sound perception such as “cochlear inner
347 hair cell degeneration” (MP:0004398), “increased or absent threshold for auditory brainstem
348 response” (MP:0011967), “cochlear ganglion degeneration” (MP:0002857), and “increased or
349 absent threshold for auditory brainstem response” (MP:0011967) (**Table 1**). We also found a
350 highly significant enrichment (adjusted $P < 4.5 \times 10^{-3}$) for the top-level mammalian PO term
351 “hearing/vestibular/ear phenotype” (MP:0005377).

352 As a control, we built null genetic models in which one of the two contrast pairs had its trait
353 status reversed, such that the echolocating dolphin and non-echolocating large flying fox were
354 treated as sharing a convergent trait, while the other two species were treated as paired
355 contrast partners. This configuration has the property that both the shared phylogenetic signal
356 and any shared convergent trait signal from the genuine trait of echolocation are canceled out.
357 Then, we applied GO and PO enrichment to the top 100 genes in the ESL-PSC models as
358 above. None of the terms in **Table 1** received significant enrichment (adjusted $P < 0.05$), as
359 expected of the null model. A recent study found that the analysis of synonymous variation can
360 help detect data contamination and other types of error ³⁶, so we developed another null test of
361 ESL-PSC by analyzing only fourfold degenerate sites expected to evolve largely neutrally in
362 mammals. No significant enrichment was found for any of the relevant ontology categories.

363 Overall, highly significant probabilities for the enrichment of hearing-related ontology terms
364 suggest that machine learning detects a strong signal of convergence in hearing-related
365 proteins in echolocators. This is the first demonstration of a multiple test-adjusted highly
366 significant signal for sound perception in a genome-wide comparative analysis of echolocation.

367

368 **DISCUSSION**

369 Discovery of genotype-phenotype relationships is of central importance in functional and
370 evolutionary genomics. Repeated evolution of the same trait in species of independent clades
371 offers an opportunity to reveal the genetic architecture shared by these independent trait
372 evolutions. We have presented a novel comparative genomics approach using machine learning
373 (ESL-PSC), informed by molecular phylogenies, to infer quantitative genetic models of trait
374 convergences. The application of ESL-PSC to two distinct, previously well-investigated
375 examples establishes that there is a significant commonality in the genetic basis of trait
376 evolution among species in independent lineages.

377 A high predictive ability of ESL-PSC was found for correctly classifying species with and without
378 C4 photosynthesis in grass clades not involved in training the model (**Fig. 4A, B**). Classical
379 molecular evolutionary methods do not commonly afford this type of quantitative prediction. The
380 high accuracy of genetic models of C4 trait evolution in which the well-studied convergent
381 protein RuBisCo was excluded is suggestive of the potential role of additional chloroplast
382 proteins in the convergent gain of C4 photosynthesis. These analyses also showed that not all
383 species with convergent traits harbor the same substitutions in the sites included in genetic
384 modes. In fact, no more than four out of six C4 species shared the same amino acid residue in

385 the sites selected during ESL model building. Therefore, ESL model building can automatically
386 extract relevant information from incomplete molecular convergence correlated with the trait
387 convergence, obviating the need to use *ad hoc* cut-offs and subsetting data by evolutionary
388 conservation^{2,3,5,7,37}. This makes ESL-PSC convergent evolution analyses less subjective and
389 more reproducible than other approaches.

390 ESL-PSC also identified genes involved in the convergent acquisition of echolocation in
391 mammals. The list of top genes in ESL models was found to be highly enriched for GO and PO
392 categories involved in auditory processes at FDR-corrected P -values that were more significant
393 than previously reported, implying that the machine learning approach to building genetic
394 models can be significantly more powerful than previous approaches. While validation of
395 ESL-PSC derived from the enrichment of functional categories is arguably circumstantial, direct
396 experimental approaches are beyond the scope of this investigation. Further support may be
397 found by assessing the potential functional relevance of the selected genes to determine
398 whether mutations in them cause diseases due to relevant functional disruptions. In the analysis
399 of Disease Ontology categories, we found a hearing-related “Sensorineural hearing loss,
400 bilateral” term to be highly enriched in the top genes (adjusted $P < 10^{-5}$). Many other terms
401 related to deafness contained a significantly greater than expected number of genes (**Table 1**).
402 No previous study has reported such an enrichment.

403 ESL-PSC appears to extract commonalities of the genetic basis of trait convergences more
404 effectively than other approaches. However, we note that species-specific evolutionary
405 substitutions may also be involved in the evolution of convergent traits. These are not the target
406 of the ESL approach and will not be included in the genetic model. Also, molecular
407 convergences in the non-coding sequences as well as regulatory innovations may be involved
408 in the evolution of convergent traits some of which may be analyzed by their simultaneous
409 analysis in the ESL-PSC framework. We plan to pursue them in the future.

410 We expect ESL-PSC to be useful as a comparative genomics tool for uncovering common
411 genetic elements involved in the evolution of traits shared between species. We envision that
412 ESL-PSC will be applied to first generate a candidate gene and site list, which can be followed
413 by a series of hypothesis tests regarding the commonality of the genetic basis of trait
414 convergences. These analyses will be extremely fast, as ESL-PSC took only minutes in most of
415 our data analyses. These results can then be followed up by conducting traditional molecular

416 evolutionary analyses and functional genomic experiments to identify selective processes at
417 play.

418

419 **References**

- 420 1. Sackton, T. B. & Clark, N. Convergent evolution in the genomics era: new insights and
421 directions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20190102 (2019).
- 422 2. Marcovitz, A. *et al.* A functional enrichment test for molecular convergent evolution finds a
423 clear protein-coding signal in echolocating bats and whales. *Proc. Natl. Acad. Sci. U. S. A.*
424 **116**, 21094–21103 (2019).
- 425 3. Lee, J.-H. *et al.* Molecular parallelism in fast-twitch muscle proteins in echolocating
426 mammals. *Sci Adv* **4**, eaat9660 (2018).
- 427 4. Liu, Z., Qi, F.-Y., Xu, D.-M., Zhou, X. & Shi, P. Genomic and functional evidence reveals
428 molecular insights into the origin of echolocation in whales. *Sci Adv* **4**, eaat8821 (2018).
- 429 5. Zou, Z. & Zhang, J. No genome-wide protein sequence convergence for echolocation. *Mol.*
430 *Biol. Evol.* **32**, 1237–1241 (2015).
- 431 6. Thomas, G. W. C. & Hahn, M. W. Determining the Null Model for Detecting Adaptive
432 Convergence from Genomic Data: A Case Study using Echolocating Mammals. *Molecular*
433 *Biology and Evolution* vol. 32 1232–1236 Preprint at <https://doi.org/10.1093/molbev/msv013>
434 (2015).
- 435 7. Xu, S. *et al.* Genome-Wide Convergence during Evolution of Mangroves from Woody
436 Plants. *Mol. Biol. Evol.* **34**, 1008–1015 (2017).
- 437 8. Kumar, S. & Sharma, S. Evolutionary Sparse Learning for Phylogenomics. *Mol. Biol. Evol.*
438 **38**, 4674–4682 (2021).
- 439 9. Parker, J. *et al.* Genome-wide signatures of convergent evolution in echolocating mammals.
440 *Nature* **502**, 228–231 (2013).
- 441 10. Yuan, Y. *et al.* Comparative genomics provides insights into the aquatic adaptations of

- 442 mammals. *Proc. Natl. Acad. Sci. U. S. A.* **118**, (2021).
- 443 11. He, Z. *et al.* Convergent adaptation of the genomes of woody plants at the land-sea
444 interface. *Natl. Sci. Rev.* **7**, 978–993 (2020).
- 445 12. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for
446 interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* **102**,
447 15545–15550 (2005).
- 448 13. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: paths
449 toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**,
450 1–13 (2009).
- 451 14. Partha, R., Kowalczyk, A., Clark, N. L. & Chikina, M. Robust Method for Detecting
452 Convergent Shifts in Evolutionary Rates. *Mol. Biol. Evol.* **36**, 1817–1830 (2019).
- 453 15. Kowalczyk, A., Partha, R., Clark, N. L. & Chikina, M. Pan-mammalian analysis of molecular
454 constraints underlying extended lifespan. *Elife* **9**, (2020).
- 455 16. Farré, X. *et al.* Comparative Analysis of Mammal Genomes Unveils Key Genomic Variability
456 for Human Life Span. *Mol. Biol. Evol.* **38**, 4948–4961 (2021).
- 457 17. Liu, Y. *et al.* Convergent sequence evolution between echolocating bats and dolphins. *Curr.*
458 *Biol.* **20**, R53–4 (2010).
- 459 18. Li, Y., Liu, Z., Shi, P. & Zhang, J. The hearing gene Prestin unites echolocating bats and
460 whales. *Curr. Biol.* **20**, R55–6 (2010).
- 461 19. Liu, Z., Qi, F.-Y., Zhou, X., Ren, H.-Q. & Shi, P. Parallel sites implicate functional
462 convergence of the hearing gene prestin among echolocating mammals. *Mol. Biol. Evol.* **31**,
463 2415–2424 (2014).
- 464 20. Christin, P.-A. *et al.* Evolutionary switch and genetic convergence on rbcL following the
465 evolution of C4 photosynthesis. *Mol. Biol. Evol.* **25**, 2361–2368 (2008).
- 466 21. Parto, S. & Lartillot, N. Molecular adaptation in Rubisco: Discriminating between convergent
467 evolution and positive selection using mechanistic and classical codon models. *PLoS One*

- 468 **13**, e0192697 (2018).
- 469 22. Casola, C. & Li, J. Beyond RuBisCO: convergent molecular evolution of multiple chloroplast
470 genes in C4 plants. *PeerJ* **10**, e12791 (2022).
- 471 23. Tibshirani, R. Regression shrinkage and selection via the lasso. *J. R. Stat. Soc.* **58**,
472 267–288 (1996).
- 473 24. Heyduk, K., Moreno-Villena, J. J., Gilman, I. S., Christin, P.-A. & Edwards, E. J. The
474 genetics of convergent evolution: insights from plant photosynthesis. *Nat. Rev. Genet.* **20**,
475 485–493 (2019).
- 476 25. Gowik, U. & Westhoff, P. The path from C3 to C4 photosynthesis. *Plant Physiol.* **155**, 56–63
477 (2011).
- 478 26. Kapralov, M. V., Smith, J. A. C. & Filatov, D. A. Rubisco evolution in C₄ eudicots: an analysis
479 of Amaranthaceae sensu lato. *PLoS One* **7**, e52974 (2012).
- 480 27. Parto, S. & Lartillot, N. Correction: Molecular adaptation in Rubisco: Discriminating between
481 convergent evolution and positive selection using mechanistic and classical codon models.
482 *PLoS One* **13**, e0196267 (2018).
- 483 28. Grass Phylogeny Working Group II. New grass phylogeny resolves deep evolutionary
484 relationships and discovers C4 origins. *New Phytol.* **193**, 304–312 (2012).
- 485 29. Besnard, G. *et al.* Phylogenomics of C4 Photosynthesis in Sedges (Cyperaceae): Multiple
486 Appearances and Genetic Convergence. *Mol. Biol. Evol.* **26**, 1909–1919 (2009).
- 487 30. Dunning, L. T. *et al.* Introgression and repeated co-option facilitated the recurrent
488 emergence of C4 photosynthesis among close relatives. *Evolution* **71**, 1541–1555 (2017).
- 489 31. Olivier Chabrol, Manuela Royer-Carenzi, Pierre Pontarotti, Gilles Didier. Detecting the
490 molecular basis of phenotypic convergence. *Methods in Ecology and Evolution* **9**,
491 2170–2180 (2018).
- 492 32. Scornavacca, C. *et al.* OrthoMaM v10: Scaling-Up Orthologous Coding Sequence and Exon
493 Alignments with More than One Hundred Mammalian Genomes. *Mol. Biol. Evol.* **36**,

- 494 861–862 (2019).
- 495 33. Davies, K. T. J., Cotton, J. A., Kirwan, J. D., Teeling, E. C. & Rossiter, S. J. Parallel
496 signatures of sequence evolution among hearing genes in echolocating mammals: an
497 emerging model of genetic convergence. *Heredity* **108**, 480–489 (2012).
- 498 34. Shen, Y.-Y., Liang, L., Li, G.-S., Murphy, R. W. & Zhang, Y.-P. Parallel evolution of auditory
499 genes for echolocation in bats and toothed whales. *PLoS Genet.* **8**, e1002788 (2012).
- 500 35. Li, G., Wang, J., Rossiter, S. J., Jones, G. & Zhang, S. Accelerated FoxP2 evolution in
501 echolocating bats. *PLoS One* **2**, e900 (2007).
- 502 36. Fukushima, K. & Pollock, D. D. Detecting macroevolutionary genotype–phenotype
503 associations using error-corrected rates of protein convergence. *Nature Ecology &*
504 *Evolution* **7**, 155–170 (2023).
- 505 37. Thomas, G. W. C., Hahn, M. W. & Hahn, Y. The Effects of Increasing the Number of Taxa
506 on Inferences of Molecular Convergence. *Genome Biol. Evol.* **9**, 213–221 (2017).
- 507 38. Liu, J., Ji, S. & Ye, J. SLEP: Sparse Learning with Efficient Projections, Arizona State
508 University, 2009. Preprint at (2011).
- 509 39. Simon, N., Friedman, J., Hastie, T. & Tibshirani, R. A Sparse-Group Lasso. *J. Comput.*
510 *Graph. Stat.* **22**, 231–245 (2013).
- 511 40. Xie, Z. *et al.* Gene Set Knowledge Discovery with Enrichr. *Curr Protoc* **1**, e90 (2021).
- 512 41. Gene Ontology Consortium. The Gene Ontology resource: enriching a GOld mine. *Nucleic*
513 *Acids Res.* **49**, D325–D334 (2021).
- 514 42. Smith, C. L. & Eppig, J. T. The mammalian phenotype ontology: enabling robust annotation
515 and comparative analysis. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **1**, 390–399 (2009).
- 516 43. Piñero, J. *et al.* The DisGeNET knowledge platform for disease genomics: 2019 update.
517 *Nucleic Acids Res.* **48**, D845–D855 (2020).
- 518 44. Motenko, H., Neuhauser, S. B., O’Keefe, M. & Richardson, J. E. MouseMine: a new data
519 warehouse for MGI. *Mamm. Genome* **26**, 325–330 (2015).

520 **Acknowledgments.**

521 The authors would like to thank Drs. Alessandra Lamarca, Jack Craig, and Sayaka Miura for
522 reading the manuscript and providing many helpful suggestions. This work was supported by
523 research grants from the National Institutes of Health to SK (R35GM139540-03) and a
524 fellowship to JA from Temple University.

525

526 **Author information.**

527 **Affiliations and authors**

528 **Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA**
529 **19122, USA**

530 John B. Allard, Sudip Sharma, Ravi Patel, Maxwell Sanderford & Sudhir Kumar

531

532 **Department of Biology, Temple University, Philadelphia, PA 19122, USA**

533 John B. Allard, Sudip Sharma, Ravi Patel & Sudhir Kumar

534

535 **Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, Japan**

536 Koichiro Tamura

537

538 **Research Center for Genomics and Bioinformatics, Tokyo Metropolitan University, Tokyo,**
539 **Japan**

540 Koichiro Tamura

541

542 **Department of Computer and Information Sciences, Temple University, Philadelphia PA,**
543 **United States of America**

544 Slobodan Vucetic

545

546 **Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, USA.**

547 Glenn S. Gerhard

548

549 **Center for Excellence in Genome Medicine and Research, King Abdulaziz University,**
550 **Jeddah, Saudi Arabia**

551 Sudhir Kumar

552

553 **Contributions**

554 S.K. conceived the idea and developed the initial method; J.A. and S.S. refined and extended
555 the method; M.S., S.S, J.A., and R.P. implemented the method; J.A. and R.P. conducted the
556 data analyses; J.A., S.K., and G.G. wrote the manuscript; all authors contributed to intellectual
557 discussions about the method and results and co-wrote the manuscript.

558

559 **Corresponding Authors**

560 Correspondence to Sudhir Kumar (s.kumar@temple.edu) and Glenn S. Gerhard
561 (gsgerhard@temple.edu).

562

563 **Ethics declarations**

564 **Competing interests**

565 The authors declare no competing interests.

566

567 **Methods**

568 Genomic alignment data retrieval and processing. Alignments of chloroplast genes were
569 retrieved from the supplemental data in ref.²². We generated translated amino acid sequences
570 from the provided nucleic acid alignments for ESL-PSC analyses. The OrthoMaM data set³² of
571 mammalian one-to-one orthologous protein sequence alignments was downloaded from
572 <https://orthomam.mbb.cnrs.fr/>. Following previous studies in which exome-scale scans for
573 convergence in echolocating mammals were performed, we analyzed echolocation in microbats
574 and toothed whales^{2-4,9,31} and used megabats and artiodactyls as non-echolocating sister
575 taxa^{2,5,6,9}. In their ESL-PSC analysis, we excluded sites containing missing data or alignment
576 gaps in individual training sets. All multiple sequence alignments (MSAs) were one-hot
577 encoded⁸, which transforms it into a numerical format that is required by the model-building
578 algorithm. The presence of the convergent trait was represented numerically by +1 and its
579 absence by -1.

580 Building Genetic Models. ESL-PSC uses the Least Absolute Shrinkage and Selection Operator
581 (LASSO)²³ logistic regression, in which coefficients are chosen to minimize a combination of the
582 difference between observed and predicted response values of the input species (the logistic
583 loss). It uses an inclusion penalty term that scales with the sum of the absolute values of the

584 model coefficients and, therefore, induces sparsity⁸. We use bilevel sparsity in which separate
585 penalties are applied for the inclusion of sites and groups of sites (e.g., proteins). The loss
586 function is minimized by gradient descent³⁸, which is re-implemented in the myESL software
587 package used for ESL-PSC implementation (<https://github.com/kumarlabgit/ESL-PSC>). We
588 estimate a new Model Fit Score (MFS) for a given genetic model, which is the root mean
589 squared difference between the input trait value (+1 and -1) and predicted trait values for all
590 species used for training the model. The best-fit genetic models have the lowest MFS value, i.e.,
591 the input and output of the genetic model are the most concordant. This is needed because
592 optimal inclusion penalties are not known beforehand in LASSO. So, the genetic model with the
593 best MFS is chosen.

594 In our analysis, the size of the penalty for each protein (group of sites) was globally controlled
595 by the inclusion penalties, but can also vary for each individual group depending on its
596 composition. Group penalties in applications of the LASSO method are typically based on the
597 square root of the number of columns belonging to the group in dataset³⁹. Applying this system
598 produced models in which proteins with fewer variable sites and lower total entropy were
599 penalized more than those with many variable sites, in the exome-wide analysis. However,
600 highly conserved proteins containing even a few variable sites can be important. Therefore, we
601 devised a penalty function for each protein in which the group penalty scales linearly with the
602 number of variable sites plus a constant equal to the median number of variable sites across the
603 proteins in the dataset (excluding fully invariant proteins). This function was effective for both
604 small-scale (chloroplast exome) and large-scale (mammalian proteome) analyses.

605 Predictive Model Ensembles. Models with similar MFS scores were combined to form
606 ensembles of models for predictions. For all model ensembles, we used a range of group and
607 site inclusion penalty values from 1%-99% of the maximum penalty that can be applied before a
608 trivial solution in which all model feature weights are set to 0 is obtained. The inclusion penalty
609 values were taken from a logspace over this range. Unless specified, we selected genetic
610 models with the best MFS or those with the top-5% MFS values.

611 Building the Candidate protein list. We estimate the Group Sparsity Score (GSS) for every
612 selected protein in every model over all inclusion penalty combinations. GSS is the sum of
613 absolute values of regression coefficients for all the selected positions in the given protein⁸. The
614 higher the GSS, the greater their importance. Proteins not included in the genetic model receive
615 GSS = 0. For every candidate gene, their overall rank is the best rank (according to their GSS)
616 they receive in any of the genetic models, with equally ranked proteins being further ordered

617 according to the maximum GSS they attained in any model. This yields an ordered list of
618 proteins whose convergent sites stand out compared with the rest of the proteome in number,
619 proportion, and strength of the concordance of their convergent site patterns with the species
620 phenotypes, without privileging any one of those considerations.

621 When each of the input species has at least one sibling species that share its phenotype for the
622 trait being studied, then different combinations of these allowable input species can be used
623 interchangeably, and models over all inclusion penalty combinations can be built for each of the
624 species combinations. The output candidate convergent proteins are then ranked by the number
625 of species combinations for which they received non-zero GSS scores in at least one model,
626 with ties being resolved by the number of species combinations in which the proteins were
627 ranked in the top 1%, followed by the highest ever rank and highest ever GSS obtained.

628 Ontology analysis. Ontology enrichment testing was performed using Enrichr⁴⁰, and *P*-values
629 were adjusted for multiple testing. Gene ontologies were obtained from GO⁴¹. We tested for the
630 biological process GO ontologies using the GO_Biological_Process_2021 set in Enrichr (6,036
631 terms). Phenotype ontologies were derived from MGI⁴². Enrichr provides PO testing using a
632 trimmed version of the MGI phenotype vocabulary. Which excludes the top three levels of PO
633 terms (4,601 terms). Disease ontologies were derived from DisGeNet (9,828 terms)⁴³. To
634 determine enrichment and overlapping genes for the top-level PO term “hearing/ vestibular/ ear
635 phenotype” (MP:0005377), we used the MouseMine⁴⁴ ontology testing tool and the
636 Benjamini-Hochberg adjustment to obtain a multiple testing adjusted *P*-value. By common
637 convention, enrichments were only considered valid if accounted for by an overlap of at least 5
638 genes. Phenotype ontology terms were retrieved from the Mouse Genome Informatics
639 mammalian phenotype vocabulary, and gene lists associated with phenotype ontology terms
640 were generated from the Mouse/Human Orthology with Phenotype Annotations (downloaded
641 from <http://www.informatics.jax.org/downloads/reports/index.html#pheno>). For gene enrichment
642 analyses, we found that it was unnecessary to use ensembles of 400 models (20 values for
643 each inclusion penalty) because the gene ranks are based on the maximum model weights
644 which do not change significantly when using a denser grid search over the space of inclusion
645 penalty. Results shown here were based on ensembles using 4 values of each inclusion penalty
646 (16 models) in each ensemble for each species combination.

647 Null Genetic Model Ensembles. There are a number of different ways to test the genetic models
648 produced by machine learning. We built null genetic models by reversing trait designations of a
649 subset of training data such that both the shared evolutionary history and shared basis of the

650 convergent trait between trait-positive species were canceled out (**Fig. 3C**). For an even number
651 $2n$ of input species contrast pairs, the largest scrambling of the input phenotype designations is
652 achieved by flipping n pairs. There are $\frac{1}{2}2^n C_n$ possible distinct null configurations. For a small n ,
653 it is possible to generate and combine all null predictions, but a random subset of possible null
654 configurations can be sampled when n is large. Another type of null model can be constructed
655 by randomly flipping (or not flipping) the residues between the two members of each contrast
656 pair at each site (**Fig. 3D**). This preserves any phylogenetic relationships present in the
657 alignment but, when averaging over a large number of such pair-randomized alignments,
658 destroys the correlations that are due to convergence. Both of these null model experiments are
659 expected to produce models whose prediction accuracy on test species not used in model
660 building is comparable to random chance. Protein lists developed by using null genetic models
661 are not expected to be enriched in any functional ontology terms beyond that expected by
662 random chance alone.

663 **Data availability**

664 Grass and mammalian protein sequence alignment data required to reproduce the analyses in
665 this article can be found at: <https://github.com/kumarlabgit/ESL-PSC>.

666 **Code availability**

667 A GitHub repository containing scripts and software used to perform the ESL-PSC analyses in
668 this study is available at <https://github.com/kumarlabgit/ESL-PSC>.

669

670

671 **Extended data**

672 **Supplementary Table 1: Echolocation ensemble model top genes**

673

Rank	Gene identifier	Ensembl accession number	GSS	# combos ranked in top 1%
1	CASQ1	ENSG00000143318	0.1659	16
2	TMC1	ENSG00000165091	0.114	16
3	ADAMTS1	ENSG00000154734	0.0766	16
4	CDH23	ENSG00000107736	0.0713	16
5	CELA1	ENSG00000139610	0.1366	16
6	GSN	ENSG00000148180	0.0628	16
7	PAH	ENSG00000171759	0.0613	16
8	TBC1D14	ENSG00000132405	0.0418	16
9	SLC26A5	ENSG00000170615	0.097	16
10	GIGYF2	ENSG00000204120	0.0914	16
11	NDRG2	ENSG00000165795	0.0694	16
12	EPYC	ENSG00000083782	0.0847	16
13	ODF1	ENSG00000155087	0.0625	16
14	HORMAD2	ENSG00000176635	0.0312	16
15	TBC1D17	ENSG00000104946	0.061	16
16	RTN4RL2	ENSG00000186907	0.0591	16
17	RIC3	ENSG00000166405	0.0514	16
18	PTCH2	ENSG00000117425	0.036	16
19	LINGO2	ENSG00000174482	0.0532	16
20	BRINP2	ENSG00000198797	0.0525	16
21	CCR8	ENSG00000179934	0.0238	16

22	DUSP2	ENSG00000158050	0.0494	16
23	EML5	ENSG00000165521	0.0236	16
24	PTBP1	ENSG00000011304	0.0514	16
25	GFM1	ENSG00000168827	0.0359	16
26	CHD1L	ENSG00000131778	0.0186	16
27	HORMAD1	ENSG00000143452	0.0469	16
28	DHX16	ENSG00000204560	0.0474	16
29	SRRM4	ENSG00000139767	0.0202	16
30	NUDCD1	ENSG00000120526	0.0294	16
31	ELOVL7	ENSG00000164181	0.0345	16
32	PHB2	ENSG00000215021	0.0437	16
33	PNPLA5	ENSG00000100341	0.0166	16
34	RHO	ENSG00000163914	0.0402	16
35	SLC38A2	ENSG00000134294	0.0217	16
36	CABP2	ENSG00000167791	0.0402	16
37	MYO6	ENSG00000196586	0.0298	16
38	RAB22A	ENSG00000124209	0.037	16
39	DDX1	ENSG00000079785	0.029	16
40	VBP1	ENSG00000155959	0.037	16
41	LPGAT1	ENSG00000123684	0.027	16
42	ARHGAP36	ENSG00000147256	0.0159	16
43	MKL1	ENSG00000196588	0.0184	16
44	PTGS1	ENSG00000095303	0.013	16
45	CHRNA9	ENSG00000174343	0.0195	16
46	MARCH6	ENSG00000145495	0.019	16

47	INTS6L	ENSG00000165359	0.0165	16
48	IRF9	ENSG00000213928	0.0115	16
49	VTA1	ENSG00000009844	0.0366	15
50	MAGEB18	ENSG00000176774	0.0191	15
51	SEMA6A	ENSG00000092421	0.0209	15
52	FAM117A	ENSG00000121104	0.0701	14
53	PECR	ENSG00000115425	0.0242	14
54	ATG7	ENSG00000197548	0.0305	14
55	ENPP7	ENSG00000182156	0.0156	14
56	PSEN2	ENSG00000143801	0.0227	14
57	PJKV	ENSG00000204311	0.0208	14
58	PER1	ENSG00000179094	0.0225	13
59	PHF20L1	ENSG00000129292	0.0274	13
60	HSPA12A	ENSG00000165868	0.048	13
61	FAM170A	ENSG00000164334	0.0283	13
62	TNS1	ENSG00000079308	0.015	13
63	LOXHD1	ENSG00000167210	0.0206	13
64	NMUR1	ENSG00000171596	0.0131	13
65	COQ9	ENSG00000088682	0.0218	13
66	YARS	ENSG00000134684	0.0241	13
67	VSIG8	ENSG00000243284	0.0204	13
68	CCSER1	ENSG00000184305	0.0174	13
69	EYA3	ENSG00000158161	0.0514	12
70	MREG	ENSG00000118242	0.0445	12
71	DTX2	ENSG00000091073	0.0307	12

72	PCYT2	ENSG00000185813	0.0411	12
73	SYNC	ENSG00000162520	0.0344	12
74	SPEF1	ENSG00000101222	0.0272	12
75	NKPD1	ENSG00000179846	0.0233	12
76	SEMA5A	ENSG00000112902	0.0155	12
77	THEM5	ENSG00000196407	0.0149	12
78	PEX11G	ENSG00000104883	0.017	12
79	MALT1	ENSG00000172175	0.061	11
80	OTUD3	ENSG00000169914	0.0586	11
81	DGKH	ENSG00000102780	0.028	11
82	HDLBP	ENSG00000115677	0.0275	11
83	GRXCR2	ENSG00000204928	0.0402	11
84	PIGQ	ENSG00000007541	0.0204	11
85	GOLGA1	ENSG00000136935	0.0127	11
86	PLTP	ENSG00000100979	0.015	11
87	MAML1	ENSG00000161021	0.0142	11
88	SOX30	ENSG00000039600	0.118	10
89	NUP160	ENSG00000030066	0.034	10
90	PLEKHG5	ENSG00000171680	0.0781	10
91	SLC26A9	ENSG00000174502	0.0371	10
92	FBLIM1	ENSG00000162458	0.0528	10
93	MRPS23	ENSG00000181610	0.0474	10
94	GPI	ENSG00000105220	0.0392	10
95	FANK1	ENSG00000203780	0.025	10
96	USB1	ENSG00000103005	0.0419	10

97	PRMT7	ENSG00000132600	0.0156	10
98	IL4	ENSG00000113520	0.02	10
99	RFX2	ENSG00000087903	0.0203	10
100	USH1C	ENSG00000006611	0.0254	10

674

675

676