Improving the performance of supervised deep learning for regulatory genomics using phylogenetic augmentation

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Structured abstract

Motivation
Supervised deep learning is used to model the complex relationship between genomic sequence and regulatory function. Understanding how these models make predictions can provide biological insight into regulatory functions. Given the complexity of the sequence to regulatory function mapping (the cis-regulatory code), it has been suggested that the genome contains insufficient sequence variation to train models with suitable complexity. Data augmentation is a widely used approach to increase the data variation available for model training, however current data augmentation methods for genomic sequence data are limited.

Results
Inspired by the success of comparative genomics, we show that augmenting genomic sequences with evolutionarily related sequences from other species, which we term phylogenetic augmentation, improves the performance of deep learning models trained on regulatory DNA sequences to predict high-throughput functional assay measurements. Additionally, we show that phylogenetic augmentation can rescue model performance when the training set is down-sampled, demonstrating that this approach improves experimental data efficiency. Overall, this data augmentation method represents a solution for improving generalization that is applicable to many supervised deep learning problems in genomics.

Availability and implementation
The open-source GitHub repository agduncan94/phylogenetic_augmentation_paper includes the code for rerunning the analyses here and recreating the figures.

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Introduction

Supervised deep learning approaches can predict chromatin accessibility[1–3], transcription factor binding[4], enhancer activity[5], and other assays from DNA sequence[6]. The performance of trained deep learning models is measured using held-out test datasets, ruling out simple overfitting to limited training datasets. Aside from demonstrating powerful predictive performance, models that learn general biological features from experimental data can lead to improved understanding of biological phenomena. Indeed, the application of various post hoc techniques[7,8] have revealed that deep learning models trained on regulatory sequences learn both known and novel motifs and motif interactions[1–5,9,10].

Using the tools of the deep learning era[11,12], computational biologists can now fit models with complexity that approaches true biochemical complexity of transcriptional regulation[13,14]. However, training models with 10^6-10^8 parameters requires large and diverse datasets, which are available only for well-studied model organisms and human cell lines. A major challenge in the field is determining how to train more complex deep learning models for applications outside of the most data-rich systems. A proposed solution is to substantially increase data volume by performing assays on randomly generated synthetic sequences, and then evaluating models trained on these sequences using true genomic sequences[13,15]. The reasoning behind this approach is that the genome does not contain sufficient variation to learn all aspects of the cis-regulatory code. Although, this approach may not be suitable for all assays and requires additional experimentation.

Data augmentation is a technique used in deep learning to increase performance of complex models by training models on transformed versions of the input data, thereby increasing the number of training examples. This technique finds application across a variety of fields including computer vision[16] and natural language processing[17]. However, there are limited domain-specific augmentation techniques for genomic sequence data. Two commonly used augmentation approaches include reverse complements[18] and genomic shifts[5,19]. Recently, sequence augmentations inspired by evolution, including point mutations, inversions, and deletions, have shown promise in improving supervised model performance and interpretability[20]. However, because these approaches introduce random sequence modifications to simulate evolution, they do not account for any functional constraint on the sequence.

Homologous sequences (or homologs for short) are DNA sequences from different species that share a common ancestor and may be under functional constraint, but have likely diverged in primary sequence[21,22]. Previously, homologs have been proposed as a form of sequence augmentation for self-supervised learning, as they can be thought of as transformed versions of their ancestral sequence that retain the same biological function (analogous to augmentations that do not alter the semantic meaning in images)[23–25]. Here, we investigate the use of homologs as a data augmentation method for supervised deep learning models that predict functional genomic assays from DNA sequence. We find that augmenting genomic sequences with homologs can improve the generalization of supervised deep learning models. Furthermore, we show that phylogenetic augmentation can restore performance on models trained on down sampled training sets, indicating that this approach has the potential to enhance data efficiency when training size is low.

Results

Phylogenetic augmentation: A method for augmenting genomic sequences using multi-species genome alignments

We define phylogenetic augmentation for supervised deep learning as the transformation of a genomic sequence from one species into a homolog from another species. This approach leverages homologs extracted from multi-species genome alignments to improve the diversity of input training data. By presenting these homologs as augmented versions of training sequences, deep learning models see a broader array of sequences during the training process.

The application of phylogenetic augmentation within supervised deep learning problems involves three phases, as illustrated in Figure 1A. Prior to model training, an initial preprocessing step is performed to extract homologs for each genomic sequence in the training set from a multi-species genome alignment that contains the species being investigated (Figure 1B). This is done before training, so that existing alignment tools can be used to extract homologs (see methods). During model training, phylogenetic augmentation is applied to all training sequences at batch generation (Figure 1C). This significantly increases the number of training examples that the model encounters. After training, the models are fine-tuned on the original genomic sequences to further improve performance[15].

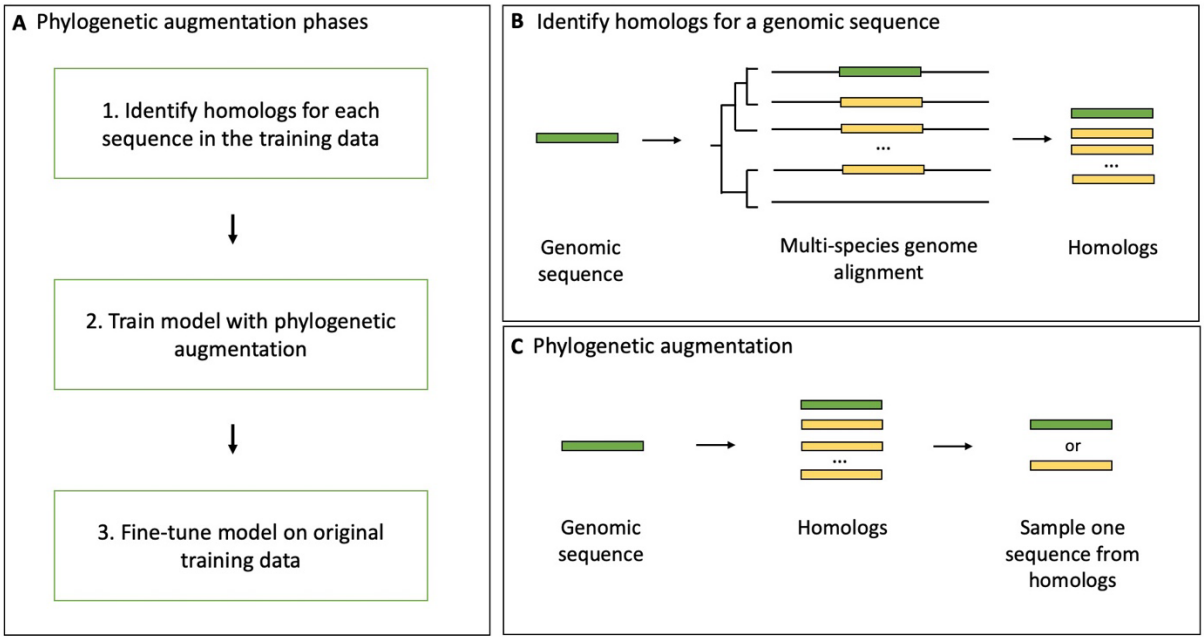

Figure 1. An overview of the phylogenetic augmentation method. (A) The three phases of phylogenetic augmentation for model training. (B) During preprocessing, homologs (yellow boxes) of each training sequence (green boxes) are identified in different genomes (black lines) using multi-species genome alignments. (C) Phylogenetic augmentation is implemented as the transformation of a genomic sequence to a random homologous sequence.

Phylogenetic augmentation improves CNN prediction performance on held-out test sets

To investigate whether phylogenetic augmentation used on a supervised deep learning problem improve model generalization, we trained convolutional neural networks (CNNs) with phylogenetic augmentation to predict *Drosophila* S2 STARR-seq activity driven by a housekeeping or developmental promoter, and compared performance to a baseline where no phylogenetic augmentation or fine-tuning was applied. For each sequence in the training set, homologs for 136 species in the *Drosophila* genus were extracted from a multi-species genome alignment. Three CNN architectures of varying complexities were trained on this data (DeepSTARR[5], ExplaiNN[9], and Motif DeepSTARR), and their performance was measured on a held-out test set (see methods).

We observed that all CNN models demonstrated an increase in test set performance compared to the baseline with the inclusion of phylogenetic augmentation and fine-tuning (Figure 2A). For

example, the average DeepSTARR model performance (Pearson correlation coefficient; PCC) increased from 0.661 to 0.689 (+4.2%) for the developmental enhancer activity, and 0.741 to 0.779 (+5.1%) on the housekeeping enhancer activity (grey and blue points). The ExplaiNN[9] CNN had a smaller performance increase than the other two architectures. This can be attributed to differences between the ExplaiNN and the DeepSTARR models. ExplaiNN uses linear combinations of learned motif representations to make predictions, and unlike the other two CNN models which have fully connected layers, cannot learn complex non-linear interactions between different transcription factor motifs[9]. While fine-tuning the models on the original training data after phylogenetic augmentation further improved performance (green points and blue points), fine-tuning the baseline models was not sufficient by itself to achieve the same level of performance increase seen with phylogenetic augmentation and fine-tuning (yellow points and blue points).

Next, we performed a similar analysis using the Basset CNN to predict binary DNase-seq peaks across 164 human cell-lines, which we refer to as the Basset dataset[1]. For every sequence in the training set, homologs from a clade of 43 species that included *Homo sapiens* were extracted from a mammalian genome alignment. Again, we observed improved model performance on a held-out test set when comparing the baseline models to the models with phylogenetic augmentation and fine-tuning, with the average area under the precision recall curve (AUPRC) increasing from 0.536 to 0.575 (+7.2%) (Figure 2B). Together, these results demonstrate that phylogenetic augmentation is a useful data augmentation approach for training supervised deep learning models on genomic sequences, though the magnitude of improvement is dependent on model architecture.

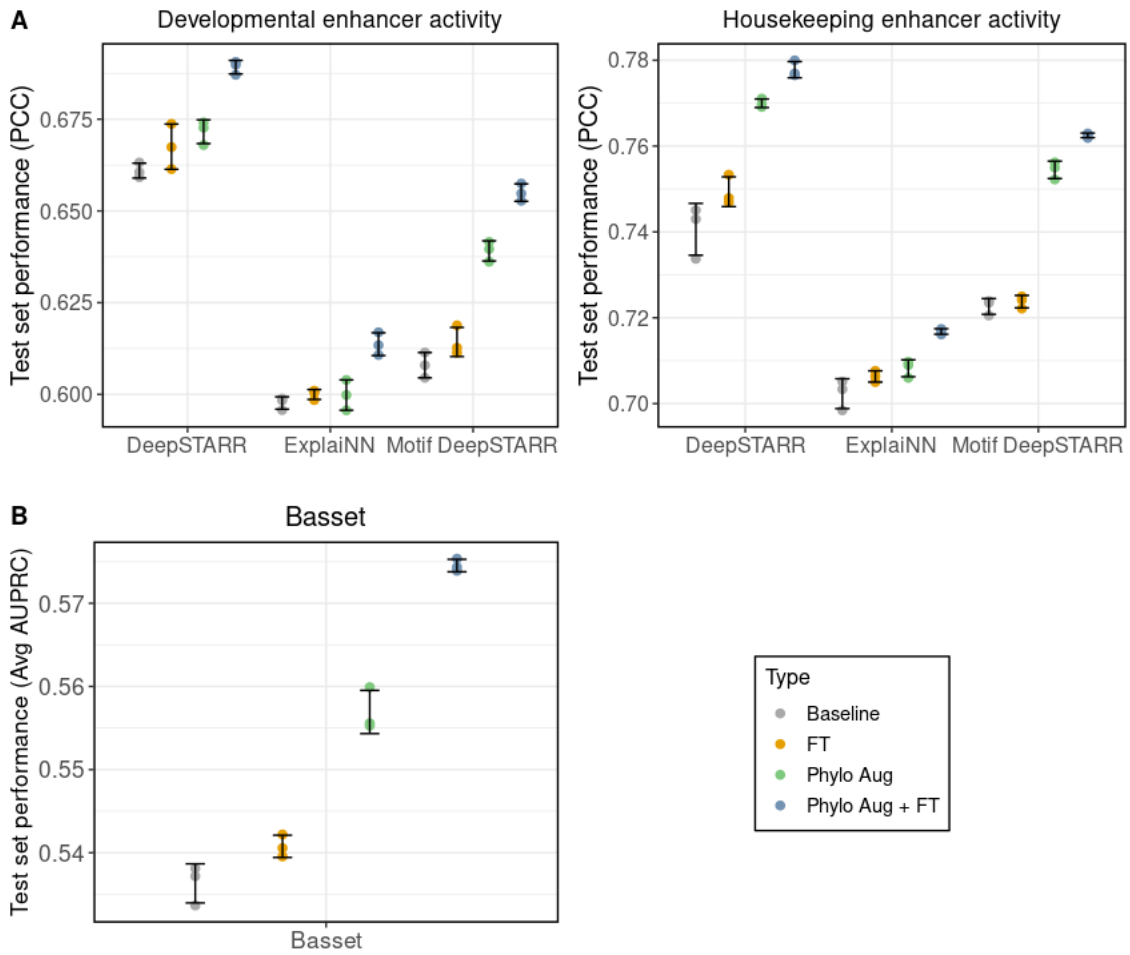

Figure 2. Phylogenetic augmentation improves model generalization with various CNN architectures. (A) CNN test performance (Pearson correlation coefficient; PCC) is shown on the y-axis for the *Drosophila* S2 Developmental (left) and Housekeeping (right) enhancer activity for trained models. (B) Basset CNN test performance (average area under the precision recall curve; avg AUPRC) is shown on the y-axis for 164 cell-type specific chromatin accessibility (DNase-seq) experiments for trained models. The grey points are the baseline models, which were trained with no phylogenetic augmentation or fine-tuning. The yellow points are the baseline models that have been fine-tuned on the original data. The green points are models trained with phylogenetic augmentation. The blue points are the models trained with phylogenetic augmentation that have been fine-tuned on the original data. The black error bars represent the standard deviation of the three replicates for each CNN architecture and dataset.

Phylogenetic augmentation improves data efficiency in supervised deep learning

Some regulatory datasets may not include enough regions of interest for effective machine learning, which could lead to overfitting and difficulty generalizing to unseen sequences. Phylogenetic augmentation could serve as a method for improving the data efficiency of smaller genomic datasets. To investigate this, we sampled different fractions of the training sequences for the *Drosophila* S2 STARR-seq dataset and applied phylogenetic augmentation during DeepSTARR model training to determine if test set performance could be rescued. For each fraction of the training sequences, phylogenetic augmentation with fine-tuning improved test set

performance compared to the baseline (blue and grey points) (Figure 3A). At 40% and 20% of the original training sequences, phylogenetic augmentation plus fine-tuning was sufficient to rescue the baseline model's performance on the test set for the developmental and housekeeping enhancer activities, respectively (dotted red lines). A similar experiment was performed for the Basset dataset using the Basset CNN, with phylogenetic augmentation plus fine-tuning again improving test-set performance for all fractions tested (Figure 3B). At around 40% of the original training data, phylogenetic augmentation was able to rescue the baseline model performance seen with all the training data. These results indicate that phylogenetic augmentation can enhance the data efficiency of supervised deep learning models, enabling them to make better predictions with less data.

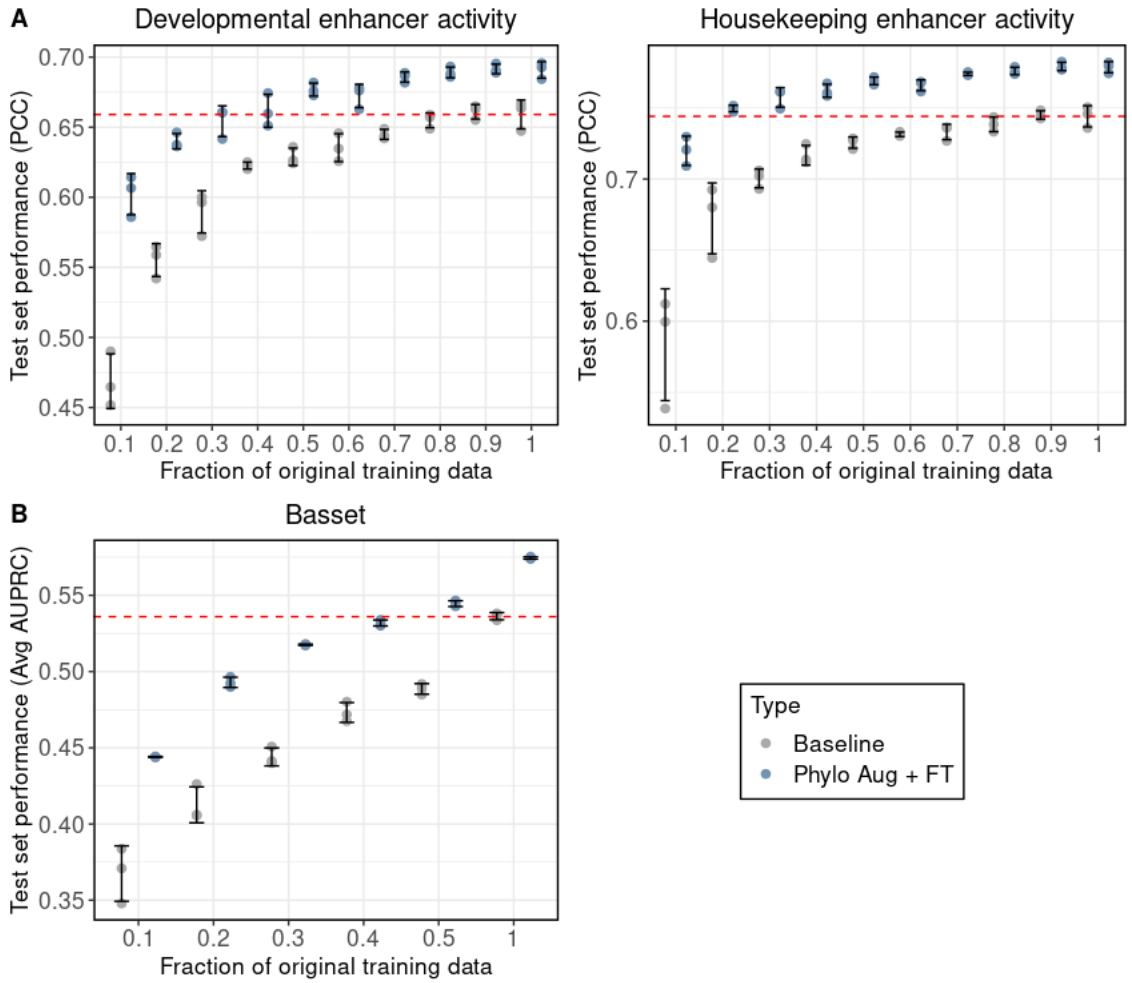

Figure 3. Phylogenetic augmentation improves data efficiency when training size is reduced. (A) DeepSTARR CNN test performance (Pearson correlation coefficient; PCC) is shown on the y-axis for the Developmental (left) and Housekeeping (right) enhancer activity for trained models. (B) Basset CNN test performance (average area under the precision recall curve; avg AUPRC) is shown on the y-axis for 164 cell-type specific chromatin accessibility (DNase-seq) experiments for trained models. The x-axis represents the fraction of the original training data that was sampled during model training. The grey points are the baseline models, which were trained with no phylogenetic augmentation or fine-tuning. The blue points are the models trained with phylogenetic augmentation that have been fine-tuned on the original data. The black

error bars represent the standard deviation of the three replicates for each CNN architecture and dataset. The dotted red line represents the average test performance of the baseline model on the original training data.

Exploring hyperparameters for phylogenetic augmentation

To assess the impact of different hyperparameters on phylogenetic augmentation, we trained multiple DeepSTARR models on the *Drosophila* S2 STARR-seq dataset and varied the number of species used and the rate at which phylogenetic augmentation was applied.

First, we examined how the number of species used during phylogenetic augmentation impacted model test performance. We arranged the species from the *Drosophila* multi-species alignment based on increasing evolutionary distance from *D. melanogaster*. Then, we trained multiple DeepSTARR models with phylogenetic augmentation, progressively incorporating more species from which to draw homologs. We observed that including homologs for just one additional species yielded a noticeable performance increase for both enhancer activity measurements, and that model test performance became saturated at around 10 species (Figure 4A). While adding additional species further improved performance for some replicates, there was higher variation among replicates. These results suggest that a handful of species is sufficient for improving model generalization through phylogenetic augmentation.

Next, we explored how the rate at which phylogenetic augmentation is applied during training affects test performance. We trained DeepSTARR models on the *Drosophila* S2 STARR-seq dataset, applying different rates of phylogenetic augmentation at batch generation. For example, a rate of 0.5 signified that 50% of the sequences in each batch underwent phylogenetic augmentation. This was carried out using homologs for all 136 *Drosophila* species from the *Drosophila* multi-species alignment. As shown in Figure 4B, an increase in the rate of phylogenetic augmentation led to improved test set performance for both enhancer activity measurements. However, past a rate of 0.5, test set performance became more variable and subsequently decreased or plateaued. These results indicate the importance of the rate of phylogenetic augmentation when using this augmentation approach, and that it is not always best to apply this augmentation to every sequence in a batch.

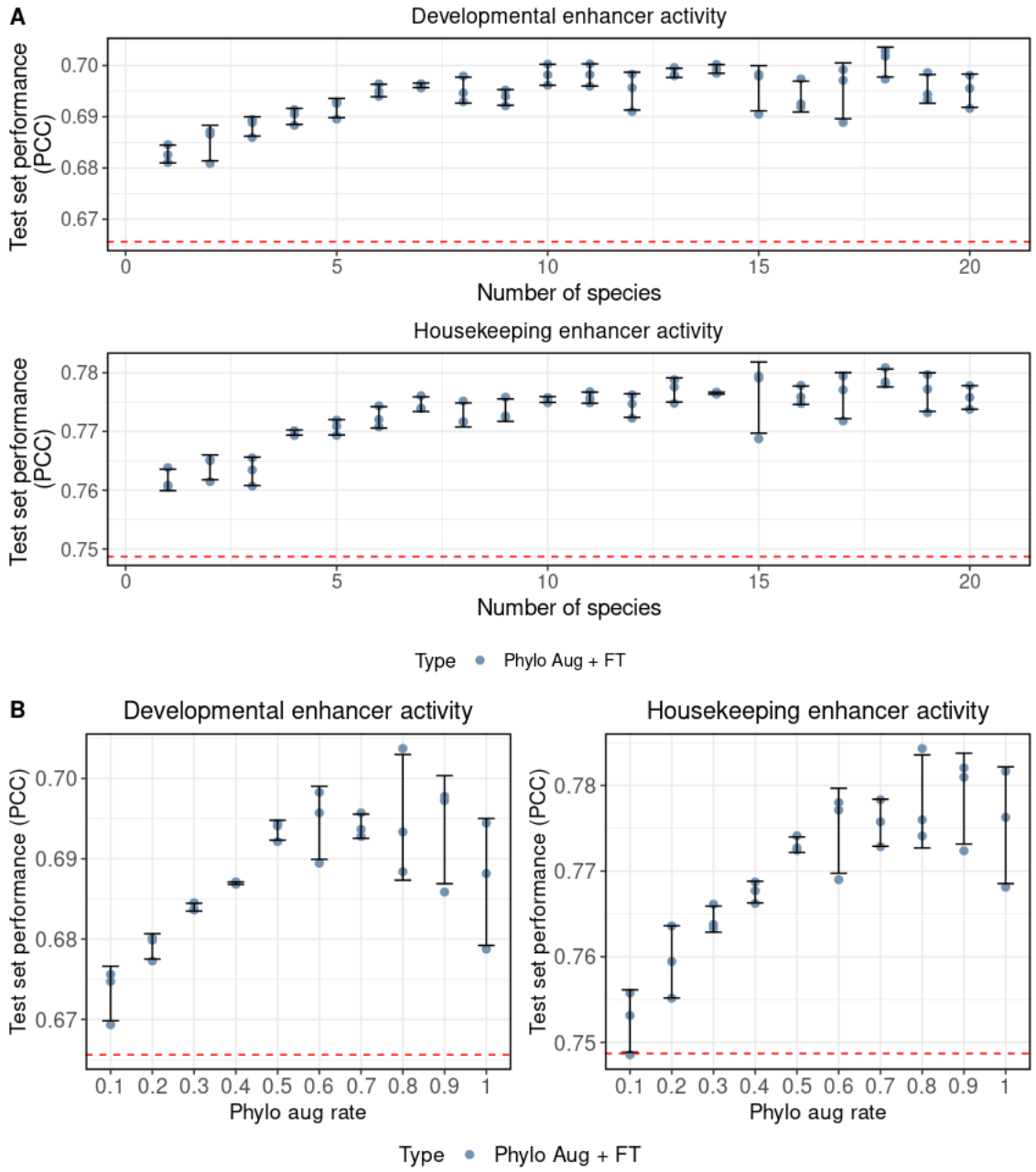

Figure 4. Hyperparameters for phylogenetic augmentation impact test set performance. (A) DeepSTARR CNN test performance (Pearson correlation coefficient; PCC) is shown on the y-axis for Developmental (top) and Housekeeping (bottom) enhancer activity for trained models. The x-axis represents the number of species included during phylogenetic augmentation. The black error bars represent the standard deviation of the three replicates that were trained for each number of species. The dotted red line represents the average test PCC on the original training data with no phylogenetic augmentation or fine-tuning. (B) DeepSTARR CNN test performance (Pearson correlation coefficient; PCC) is shown on the y-axis for Developmental (left) and Housekeeping (right) enhancer activity for trained models. The x-axis represents the rate at which phylogenetic augmentation is applied during model training. The black error bars represent the standard deviation of the three replicates that were trained for each rate. The dotted

red line represents the average test PCC on the original training data with no phylogenetic augmentation or fine-tuning.

Discussion

Here, we introduced a data augmentation method for supervised deep learning of genomic sequences that takes advantage of multi-species genome alignments and the phylogenetic relationship between homologous sequences. This approach improved the generalization and data efficiency of a regression and a classification problem for two different functional genomic datasets, suggesting it is a general approach for augmenting genomic sequence data. Additionally, we used phylogenetic augmentation on a reduced dataset to rescue model performance seen with the original training dataset, demonstrating that this approach improves the data efficiency of models.

Regulatory elements, like promoters and enhancers, undergo evolution at different rates, which can lead to varying rates of element turnover[26]. Thus, the efficacy of this approach can be influenced by the species chosen for augmentation, as some elements may lose function earlier than others. Since the *Drosophila* S2 STARR-seq data contains enhancers, which have higher rates of turnover, we restricted species to those in the *Drosophila* genus. Even within the *Drosophila* genus, some species only had a minor proportion of homologs corresponding to the *D. melanogaster* sequences. Had we included only species outside of the genus, such as other insects, the number of homologs identified would have been noticeably lower. Consequently, the phylogenetic augmentation method likely would have had a decrease in effectiveness. An understanding of the biology of the dataset being studied will guide researchers using this augmentation approach to choose the most suitable species.

In machine learning, data augmentation finds common use when datasets are too small such that deep learning models will memorize or overfit the training data. Although many regulatory datasets, such as those resulting from functional genomic experiments, contain large quantities of data, not all do. For instance, curated databases like the VISTA enhancer database include only a few thousand mouse and human enhancers[27]. Potentially, data augmentation approaches like phylogenetic augmentation could contribute to addressing the challenge of training deep learning models on these small genomic datasets.

As researchers are increasingly relying on the features learned by deep learning models trained on sequence data to obtain biological insights of regulatory function, it is crucial that these models are not overfitting to their training data and generalize to unseen sequences. The method we proposed here holds the potential for application in any supervised deep learning problem that uses non-coding genomic sequences as input. With the increasing availability of large alignments such as the 240-way mammalian alignment from Zoonomia[22], the 341-way avian alignment from B10K[28], and the 168-way *Drosophila* alignment used here, our approach is widely applicable across a variety of species.

Methods

Datasets

We used published data from a massively parallel reporter assay (MPRA) experiment measuring enhancer activity across the *Drosophila Melanogaster* genome in the S2 cell-line[5]. The MPRA was performed once using a housekeeping promoter and once with a developmental promoter to investigate enhancer-promoter dependencies, resulting in two measurements per sequence. The dataset consists of 7,062 and 11,658 enhancers identified in the housekeeping and developmental experiments, respectively. Additionally, 223,306 regions with varying activity levels were also included to provide sequences with a range of activity levels. All sequences are of length 249bp. To match the original manuscript, chromosome 1 was split in half, with the first half used for validation and the second half used for testing. The remainder of the data was used for model training. Instead of including reverse complements in the dataset, reverse complements were randomly applied during model training to 50% of sequences.

The Basset dataset contains 2,071,886 DNase-seq peaks from 164 different human cell-lines, along with a binary class for whether each peak is open in each cell-type. After filtering out sequences with Ns (unknown nucleotides), this was reduced to 2,021,532 regions. All sequences are of length 600bp. To match the original manuscript, the data was split into training, validation, and testing using 93%, 3.5%, and 3.5% of the data, respectively.

Models

For the *Drosophila* S2 analysis, we used three different CNN architectures designed for DNA sequences and implemented them in TensorFlow: DeepSTARR[5], ExplaiNN[9], and a more interpretable version of DeepSTARR that we call Motif DeepSTARR. These architectures represent various levels of model complexity and interpretability. The input to these models is 249bp DNA sequences that are one-hot encoded, and the task is a regression on the MPRA enhancer activity for each promoter MPRA experiment. A summary of the encoder component of the architectures is provided below.

- **DeepSTARR**: A CNN composed of four convolutional layers followed by two fully connected layers. The convolutional layers are meant to capture sequence motifs, and the fully connected layers are meant to capture non-linear relationships between motifs.

- **Motif DeepSTARR**: A single convolutional layer with 256 filters, followed by two fully connected layers. The convolutional layer has a large filter size (19) in order to learn interpretable motifs. The fully connected layers capture non-linear relationships between motifs.

- **ExplaiNN**: A linear combination of 256 motif representations. The motif representations are defined as a convolutional layer with 1 filter, followed by two fully connected layers.

For the Basset analysis, we used only the Basset CNN[1]. The input to this model is 600bp DNA sequences that is one-hot encoded, and the task is 164 multi-label binary classification on164 different human cell-lines. A summary of the encoder component of the architectures is provided below.

- **Basset**: A CNN composed of three convolutional layers followed by two fully connected layers. The inspiration for the DeepSTARR architecture.

Identifying homologous sequences from a multi-species genome alignment
For the *Drosophila* S2 analysis, we obtained an unpublished multi-species genome alignment of 168 genomes containing 137 species from the *Drosophila* genus, including *D. melanogaster* (Supplemental data file 1). Homologs from other *Drosophila* species were extracted from the alignment for each *D. Melanogaster* training sequence using HAL[29] and the HALPER tool[30]. If multiple homologs were found in a target organism, the first match was arbitrarily selected. The selected sequences were then resized to 249bp from the midpoint to match the length of the training sequence. This resulted in 10,582,901 homologs in total across all 191,109 sequences in the training set.

For the Basset analysis, we used the Zoonomia multi-species genome alignment of 241 mammalian species[22]. Homologs from 42 species were used for this analysis and were restricted to clades around the *Homo sapiens* node (Supplemental data file 2). The selected sequences were then resized to 600bp from the midpoint to match the length of the training sequence. This resulted in 62,538,898 homologs in total across all 1,880,029 sequences in the training set.

Model training and fine tuning
For the *Drosophila* analysis, models were trained using TensorFlow[11] with the Adam optimizer[31] for 100 epochs with a learning rate of $2x10^{-3}$, mean squared error loss, and early stopping of 10. During model training, phylogenetic augmentation was applied to each training sequence. This was done by sampling from the set of homologs for a given training sequence. A reverse complement augmentation was also applied randomly to sequences, including when no phylogenetic augmentation was used. As in a recent study of sequence augmentations, after training the models using the phylogenetic augmentation approach, we fine-tuned for 5 epochs at a lower learning rate ($1x10^{-4}$) using only the original training sequences[20]. Phylogenetic augmentation is never applied to the validation or testing sets. For the sampling experiment, we trained the DeepSTARR CNN with sampled fractions of the training data. Sampling was performed separately for each replicate. We use Pearson Correlation Coefficient (PCC) to measure each model's performance for each task on the same held-out test dataset.

The Basset models were trained similarly to the *Drosophila* analysis, with the following changes. Models were trained with the Adam optimizer for 20 epochs with a learning rate of $2x10^{-3}$ and binary cross entropy loss. The average area under the precision recall curve (AUPRC) was used to measure the model's performance for each task on the held-out test dataset. This was used in place of the average area under the curve (AUC), as the data has a large class imbalance between positives and negatives.

Investigating hyperparameters
For the number of species analysis, the *Drosophila* phylogenetic tree was extracted from the *Drosophila* multi-species Cactus alignment[32] using the halStats command (--tree) from the HAL package[29]. The ETE Toolkit[33] get_distance function was used to determine the distance between *D. melanogaster* and all other species in the phylogenetic tree. These species were then sorted by

their ascending evolutionary distance. The DeepSTARR model was then trained using phylogenetic augmentation on the homologs for the closest species, then the two closest species, and so on. For each set of species, three DeepSTARR replicates were trained.

For the phylogenetic augmentation rate analysis, the DeepSTARR model was trained on the *Drosophila* S2 dataset using increasing rates of phylogenetic augmentation. Homologs for all 136 *Drosophila* species from the alignment were used. Three replicates were run for each rate of augmentation.

Acknowledgements
We thank Cameron Dufault, Nawrah Khader, Denise Le, Shanelle Mullany, Ami Sangster, and Tiegh Taylor for comments on the manuscript. We would also like to thank Dr. Bernard Kim for sharing the 168-way *Drosophila* genome alignment.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC 2020-05972, operating grant held by J.A.M. and an NSERC discovery grant to A.M.M) This research was performed on infrastructure obtained with grants to A.M.M from the Canada Foundation for Innovation.

Data availability
The data underlying this article are available on Zenodo. The *Drosophila* alignment is available upon request from Dr. Bernard Kim (Stanford University).

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