Leveraging the k-Nearest Neighbors Classification Algorithm for Microbial Source Tracking Using a Bacterial DNA Fingerprint Library

Jeffrey D. McGovern
Department of Computer Science
California Polytechnic State University
San Luis Obispo, CA 93407
Email: jmcgover@calpoly.edu

Alexander Dekhtyar
Department of Computer Science
California Polytechnic State University
San Luis Obispo, CA 93407
Email: dekhtyar@calpoly.edu

Christopher Kitts
Department of Biological Sciences
California Polytechnic State University
San Luis Obispo, CA 93407
Email: ckitts@calpoly.edu

Michael Black
Department of Biological Sciences
California Polytechnic State University
San Luis Obispo, CA 93407
Email: mblack@calpoly.edu

Jennifer VanderKelen
Department of Biological Sciences
California Polytechnic State University
San Luis Obispo, CA 93407
Email: jvanderk@calpoly.edu

Anya Goodman
Department of Biological Sciences
California Polytechnic State University
San Luis Obispo, CA 93407
Email: agoodman@calpoly.edu

***Abstract*—Fecal contamination in bodies of water is an issue that cities must combat regularly. Often, city governments must restrict access to water sources until the contaminants dissipate. Sourcing the species of the fecal matter helps curb the issue in the future, giving city governments the ability to mitigate the effects before they occur again. Microbial Source Tracking (MST) aims to determine source host species of strains of microbiological lifeforms and library-based MST is one method that can assist in sourcing fecal matter. Recently, the Biology Department in conjunction with the Computer Science Department at California Polytechnic State University San Luis Obispo (Cal Poly) teamed up to build a database called the Cal Poly Library of Pyroprints (CPLOP). Students collect fecal samples, culture and pyrosequence the *E. coli* in the samples, and insert this data, called pyroprints, into CPLOP. Using two intergenic transcribed spacer regions of DNA, Cal Poly biologists perform studies on strain differentiation. We propose using k-Nearest Neighbors, a straightforward machine learning technique, to classify the host species of a given pyroprint, construct four algorithms to resolve the regions, and investigate classification accuracy.**

I. Introduction

Microbial Source Tracking (MST) aims to determine the source host species of strains — genetic subtypes — of microbes. Be it through the makeup of different microorganisms or the presiding strains of a particular microorganism in the source matter — fecal matter — researchers measure strain characteristics that distinguish source matter between different species. MST is a necessary step in a variety of applied studies: for example in order to properly address the issue of fecal contamination in a water source, identifying the root cause, i.e., the the species that causes of the contamination, is key.

Fecal provenance is one use for MST that attempts to delineate the strains of bacteria that reside within each species. In recent years biologists conjectured that the bacterial strains of fecal indicator bacteria, such as *Escherichia coli* (*E. coli*) are usually specific to the species, outside of a certain percentage of so-called *transient strains*. Strain identification and sourcing allows researchers to characterize the species of provenance of the source matter that the microbes inhabit.

A common method of MST starts with collecting fecal matter from a known host. Next is to culture isolates from relevant microbes in the fecal matter, obtain a strain-level digital representation of each collected isolate, and store such representations in a database to create a digital collection of isolates with known sources. The physical isolates collected are referred to as a library, while their digital representations are contained within a database. The data inserted into such a database can range from collection information about the microbiome, to a particular microbe characterization, or to any other useful set of metrics that can appropriately profile an entry [4].

Following the creation of a microbial isolate database, an MST method proceeds as follows. Researchers take an environmental sample with an unknown source and process the microbial isolates using the same procedure as the known-source-isolates in the library. The strain representation obtained in the result is compared to the known strain representations in the database and any close matches are found. Since the source of the isolates in the database is known, biologists can make appropriate decisions regarding the source of the isolates in the environmental sample [7].

In 2011, the Biology Department and Computer Science Department at California Polytechnic State University San Luis Obispo (Cal Poly) developed a pyrosequencing-based method for bacterial strain identification. Based on this method, they assembled a library of *E. coli* isolates collected from a variety of known host species, called The Cal Poly Library of Pyroprints (CPLOP) [1][1], and built a corresponding database of digital fingerprints. It has supported numerous research

[1] http://www.cplop.org

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projects related to collection and analysis of *E.coli* as it occurs in host species and in the environment. However, while the original purpose of CPLOP was to support MST and many MST studies have been conducted, up until this paper, none have been published.

This paper describes our first published Microbial Source Tracking study using CPLOP. We use a slightly modified k-Nearest Neighbors classification method [2] to answer the following question: *for what percentage of CPLOP isolates can we properly identify the host species?* There are a variety of approaches one can take to answer this question. The k-Nearest Neighbors method is simple and straightforward: we elected to use it in our first study because it provides a nice baseline for all followup improvement attempts.

The main contributions of the paper are as follows:

- We modify the k-Nearest Neighbors method by adding one more parameter: the threshold α of similarity between the neighbors, beyond which no new neighbors are used. As such, the version of k-Nearest Neighbors used in this paper is the intersection of the regular nearest neighbor construct with a range query.
- The pyroprint representations of bacterial isolates give rise to multiple similarity scores between a pair of isolates. We describe a number of ways in which these multiple scores can be combined into a single decision procedure to select the "winner". In our study, we determine which of the ways to combine the similarity scores (we call the resolution methods) works best.
- We report on our empirical study to determine the accuracy of determining the host species for the bacterial isolates whose pyroprint representations are stored in CPLOP. In the study, we look at the best values of k (number of nearest neighbors to compare to), threshold α, as well as compare the results based on the four similarity score resolution methods.

The rest of the paper is organized as follows. Section II introduces CPLOP and the pyroprinting process. Section III describes the variant of k-Nearest Neighbors with thresholding we used in the study and the similarity resolution techniques we used. Section IV shows the results of the study, and Section V concludes with the discussion of our results.

II. CPLOP: The Cal Poly Library of Pyroprints

CPLOP stores information about multiple collected bacterial isolates of *E. coli*. The information stored in CPLOP is called *pyroprints* [1]: the peak heights of pyrosequences of specially constructed DNA products extracted from the *E. coli* DNA. In what follows we provide a brief description of the pyroprinting process and the CPLOP data.

Pyrosequencing is a DNA sequencing technique appropriate for sequencing short DNA fragments (up to around 150-200 base pairs). [5] While pyrosequencing does not produce the exact sequence of nucleotides, it is significantly less expensive than methods that do, with a single pyrosequencing run costing on the order of tens of dollars (not counting the cost of equipment). A pyrosequence of a DNA fragment is represented as a vector of real values — one value per dispensed nucleotide, indicating the intensity of light emission that occurred during the sequencing reaction (light is emitted in response to a specific nucleotide reagent used in the sequencing process). *Pyroprinting* is a fingerprinting technique for bacteria that uses pyrosequencing to sequence a "mixed" DNA product, such that the resulting pyrosequence cannot be used to reproduce the DNA sequence, but instead becomes a "fingerprint" of the mixed product [1].

Depicted in Figure 1 is an abstracted segment of *E. coli* DNA used in CPLOP. *The shown pattern repeats around the ring of E. coli DNA seven times*. The internal transcribed spacers (ITS) between the *16S*, *23S*, and *5S* ribosomal DNA regions each contain non-coding DNA. These segments of DNA offer keen insight into strains of *E. coli*. Since they are non-coding, random variations occur that do not affect the survivability or reproducibility of the microbe. Since any offspring of a given *E. coli* strain inherits this DNA, the *16S-23S* and *23S-5S* regions offer the ability to differentiate strains [6].

We pyroprint each ITS region separately. The DNA product that becomes sequenced *is a a PCR-amplified mix of the DNA from the seven loci of the ITS region in the E. coli DNA*. Each locus has a DNA sequence that may be different from the sequences in the other six loci, but all seven loci can be amplified jointly by selecting appropriate primers [1]. We refer to pyroprints from these regions as *16S-23S* and *23S-5S*.

Fig. 1. A diagram of a simplified segment of *E. coli* DNA, outlining the *16S-23S* and *23S-5S* ITS regions.

Each species recorded in the database has a collection of hosts, which are individual instances of a species. A host is the animal from which biologists retrieved the fecal sample. Since biologist observed these hosts producing the fecal sample, we know the source species of each fecal sample obtained. From each host, biologists create isolates.

Isolates are the cultured samples of *E. coli* from a host's fecal sample. Once a fecal sample is collected, bacterial cultures are grown from it and preserved. Each isolate undergoes PCR procedures that amplify the DNA in the two ITS regions, after which the pyrosequencing of each region produces pyroprints which are stored in CPLOP.

Pyroprints are CPLOP's most instrinsic entry. Each pyroprint comes from an isolate. Each isolate has at least one pyroprint in each *16S-23S* and *23S-5S* ITS region. Hosts might have multiple isolates and each species we consider has multiple hosts.

III. Classification Methodology

In order to understand how useful CPLOP is for MST studies, we need to determine whether the data currently stored in CPLOP can be used to properly identify the host species of a pyroprinted isolate. A sufficiently straightforward approach to such an initial study is to use the part of CPLOP that stores isolates that came from known host species as both the training

set and the test set, and determine if we can replicate the correct identification of the host species [3][1].

For our study, we use a modified version of the k-Nearest Neighbors classification method, which uses host species names as category labels. The similarity between the isolates is computed by comparing isolate pyroprints from the same ITS region using Pearson Correlation Coefficient. These similarity scores are combined using four different resolution procedures to produce the resultant category label. These three components of the process are described below.

A. Pearson Correlation

As mentioned in Section II, a pyroprint of a specific ITS region of a bacterial isolate is a vector $p = (x_1, \ldots, x_i)$ where, $i \in \mathbb{N}$ is called a *dispensation* - the position in the pyrosequencing process when the i^{th} nucleotide was dispensed, and $x_i \in \mathbb{R}$ is a numerical characteristic of the i^{th} dispensation. CPLOP uses *peak heights*, the highest light emission values registered during the i^{th} dispensation, as the values x_i of such characteristics.

For comparing two pyroprint vectors, we use the Pearson Correlation as defined in Definition III.1. Since it is the random variation in the DNA regions that create the light values in each vector, Pearson Correlation lends itself well toward our ends.

Definition III.1. Suppose we have two vectors, $\vec{u}, \vec{p} \in \mathbb{R}^n$. The Pearson Correlation is a mapping, $S : \mathbb{R}^n \times \mathbb{R}^n \to [0, 1] \subset \mathbb{R}$:

$$S(\vec{u}, \vec{p}) = \frac{\sum_{i=1}^{n}(u_i - E(\vec{u}))(p_i - E(\vec{p}))}{\sqrt{\sum_{i=1}^{n}(u_i - E(\vec{u}))^2}\sqrt{\sum_{i=1}^{n}(p_i - E(\vec{p}))^2}} = \frac{cov(\vec{u}, \vec{p})}{\sigma_{\vec{u}} \cdot \sigma_{\vec{p}}} \tag{1}$$

where $E(\vec{p})$ is the mean of $\vec{p} = (p_1, \ldots, p_n)$, $cov(\vec{u}, \vec{p})$ is the covariance of $\vec{u}$ and $\vec{p}$, and $\sigma_{\vec{p}}$ is the standard deviation of $\vec{p}$.

In essence, the Pearson Correlation is the variance between two vectors normalized by the standard deviation of each. This provides the following properties:

For $\vec{u}, \vec{p}$ and S defined in Definition III.1:

- $S(\vec{p}, \vec{p}) = 1$
- $S(\vec{u}, \vec{p}) = S(\vec{p}, \vec{u})$
- Dissimilar $\vec{u}$ and $\vec{p}$ have $S(\vec{u}, \vec{p})$ close to 0

B. k-Nearest Neighbors (with α Threshold)

The k-nearest neighbors classification algorithm is a straightforward algorithm to classify an unclassified object using a library. We use the concept of a comparison metric to formulate an idea of "closeness." To outline the process:

Given an unclassified object U, a library of classified objects $\mathbb{L}$, and a comparison metric, c:

1) Compare U to each object in $\mathbb{L}$ using c
2) Add the classified object and the result to a list of neighbors, N
3) Sort N by most similar
4) Consider only the top k entries in N, called the k-nearest neighbors
5) Classify U as the *most plural* classification in the k-nearest neighbors list

The motivation is that the unclassified object must be "close" to some of the classified objects in our database, using an appropriate measure of closeness for the data. By choosing the "most plural" classification — the classification that shows up the highest number of times — in the k-nearest neighbors we can, with some accuracy, classify our unknown object.

Our first modification to k-nearest neighbors is an additional condition at step 4:

4) Consider only the top k entries in N above threshold α

The α threshold allows biologists to filter out neighbors that are among the k closest, but too dissimilar to compare. When comparing multiple pyroprints of the same region of a single isolate, the Pearson Correlation between them is strictly above 0.99. As a result, for many other studies — not necessarily MST-focused — a Pearson Correlation of 0.99 or above is used to define a strain of *E. coli*. Filtering by some value near this may give more accurate results and provides an intuitive way to relate these lists to other studies.

C. Comparing Isolates

Of primary interest to the biologists using CPLOP is comparing isolates to each other. In CPLOP, each isolate is represented by a pair of mutually incomparable pyroprints: one for each of the two ITS regions. As a result, given isolates I_1, I_2, we can represent each as a pair of pyroprint vectors

$$I_1 = (\vec{q}_1, \vec{q}_2) \text{ and } I_2 = (\vec{r}_1, \vec{r}_2),$$

where $\vec{q}_1$ and $\vec{r}_1$ are respectively I_1 and I_2's *16S-23S* pyroprint and $\vec{q}_2$ and $\vec{r}_2$ are respectively I_1 and I_2's *23S-5S* pyroprint [1]. Since pyroprints from different regions are incomparable, comparing isolates must be done as follows:

$$c(I_1, I_2) = (sim(\vec{q}_1, \vec{r}_1), sim(\vec{q}_2, \vec{r}_2)),$$

where sim is between pyroprints and is the Pearson Correlation. Thus, when comparing isolates, we effectively have two different similarity metrics, one for each region:

$$c(I_1, I_2) = (c_1(I_1, I_2), c_2(I_1, I_2)).$$

In order to accommodate these separate similarity metrics, we need a resolution procedure. Rather than creating a new similarity metric out of a pair of similarity scores, we choose to update the k-Nearest Neighbors method with four different ways of selecting the resultant category label based on how the pyroprints compare to each other. These four methods are described below.

In what follows, we generalize our problem. Given U and V, two library objects (isolates), and a collection of comparison metrics, $\mathbb{C}= (c_1, \ldots c_m)$, with $m > 1$, comparing U to V gives us a collection of values: $c(U, V) = (c_1(U, V), \ldots, c_m(U, V))$. All four resolution procedures described below work with such a generalized representation of isolates and comparison metrics between them.

Given an unknown isolate U, a library of classified[2] isolates $\mathbb{L}$, and a set of comparison measures $\mathbb{C}$, we compare U to each object in $\mathbb{L}$ using each comparison metric in $\mathbb{C}$. To resolve these comparison metrics, we propose four algorithms:

1) Meanwise Resolution: For U and a $P \in \mathbb{L}$, we take the mean of the result of all of the comparison metrics and build a single k-nearest neighbors list from it. The mean can be any metric mapping $\mathbb{R}^n \times \mathbb{R}^n \to \mathbb{R}$ and in the investigated implementation, we use the euclidean distance, also known as the L^2 norm. A single k-nearest neighbors list results from this algorithm that we filter by k and α and use to classify the unknown.

2) Resolution by Winner: For each comparison metric, we make a k-nearest neighbors list and filter by k and α accordingly. Once we finish building each comparison metric's k-nearest neighbors list, we find the most plural classification from each list and track the number of times that classification shows up in that list. Then, we classify u based off the classification that has the highest number in its corresponding list.

3) Resolution by Union: For each comparison metric, we make a k-nearest neighbors list and filter by k and α accordingly. After building each k-nearest neighbors list, we combine the lists into a set, keeping track of the original list position for tie-breaking. From this set, which we dub the union, we count the classifications present in the union and classify u as the most plural in the union of the lists, compared to the other lists.

4) Resolution by Intersection: For each comparison metric, we make a k-nearest neighbors list and filter by k and α accordingly, but ensure that we do not lose track of the entire sorted list of results. After building each k-nearest neighbors list, we inspect each list for common isolates. We add isolates that appear in every list into a set that we call the intersection. If the size of the intersection is k, then we are done. Otherwise, we increase the length of our individual lists by δ and search for common isolate. This process repeats until the size of the intersection is k, or all of the isolates in the individual lists are below threshold α.

D. Cross Validation with Holdout

To gauge accuracy of the results, we cross-validated against the library by separately holding out each isolate in CPLOP from CPLOP, classifying it against CPLOP, and verifying whether it is correct. Since each isolate in CPLOP has the correct species, we know whether a classification is correct or not.

E. Library Makeup

CPLOP contains data from many different studies. Some investigate *E. coli* strain similarities and differences between species. Others are longitudinal, focusing on the change in *E. coli* strains within the same host. Some are even a mixture of the two, looking at the change in *E. coli* strains as a host of one species is exposed to the host of another species. Table I shows the breakdown of how many hosts, isolates, and pyroprints a given species has for the dataset we used to validate these algorithms.

We validated these algorithms using most of this data, filtering out pyroprint and species according to the following criteria:

- Pyroprints of environmental sources, such as lakes, rivers, and oceans.
  - Our focus of this study is to gauge the accuracy of animal species. Future studies will look into environmental sources and how animal species contribute to *E. coli* strains within.
- Pyroprints flagged as erroneous
- Isolates that no longer have any pyroprints in either the *16S-23S* or *23S-5S* ITS region
  - Our focus is on how well the resolution between two ITS regions work. Results focusing on the edge case of missing regions may be investigated in the future.
- Species with 3 or fewer isolates
  - Our motivation is that a species with 3 or fewer isolates would have difficulty building a majority in most k lists. Furthermore, we understand that there are problems with underrepresented species in our library and prefer to investigate well represented species.

Data removed according to the above criteria is not shown in Table I. One small note is that there may have been multiple species entries due either to typos, or nomenclature differences between studies. In Table I, we merged these different named species into the most familiar species name and counted accordingly.

IV. Results & Evaluation

There are a few areas of focus that we have when interpreting the results:

- What size k achieves the best results?
- What size α achieves the best results?
- Which metric resolution algorithm achieves the best results?

A. Evaluation Metrics

Indeed we can define "best" in many ways, but we choose to look at two metrics, recall and precision, and a combination of the two, the F-measure. The metrics look at the accuracy of the classification on the object and the object on the classification respectively, while F-measure hopes to represent a balance between the two.

1) Recall: In our study recall tracks how well we are able to discover all isolates from a given category, i.e., with a given host species. Given a category/host species name, the recall for that host species is the percentage of isolates taken from this host species that have been properly identified. For example, if our database had 100 cat isolates, and 74 of them were classified by our method as having come from a cat, the

[2]A "classified isolate" is an isolate for which the host species has been identified in the database.

TABLE I. A BREAKDOWN OF THE SPECIES REPRESENTED IN CPLOP, AND THE NUMBER OF HOST, ISOLATE, AND PYROPRINT ENTRIES FOR EACH.

Species Name	Number of hosts	Number of isolates	Number of pyroprints
Barn Owl	3	5	13
Bat	1	37	74
Bear	1	6	12
Bobcat	1	4	8
Sea Lion	3	6	12
Cat	36	39	91
Chicken	15	40	82
Cliff Sparrow	14	28	59
Common Loon	2	4	8
Cow	427	1838	3772
Coyote	2	4	8
Deer	2	20	40
Dog	50	269	573
Elephant Seal	2	4	8
Great Horned Owl	2	4	11
Grey Fox	1	4	8
Ground Squirrel	50	196	401
Horse	49	51	102
Human	227	1590	4189
Mountain Lion	5	32	64
Opossum	5	12	24
Pelican	3	8	18
Pig	32	66	149
Pigeon	107	194	515
Rabbit	1	4	8
Racoon	2	4	9
Red Tailed Hawk	2	5	11
Red-shoulder Hawk	2	4	11
Sea Otter	3	10	20
Seagull	31	11	22
Sheep	50	94	200
Sparrow	8	15	30
Wild Turkey	36	72	177
Total species: 32	1236	4682	10732

recall would be 74%. In this study, we compute both overall recall (what percentage of isolates were classified as their proper label) as well as species-level recall (what percentage of isolates that came from dogs/humans/sheep/etc. were classified as their proper label).

2) Precision: Precision tracks how well our method avoids misclassification errors. Given a category and a list of isolates our method classified as belonging to it, the precision of the method on the category is the percent of isolates from the list that has the correct label. For example, if our method returned 100 isolates labelled "Dog" of which 77 isolates really did come from dogs, the precision of the method is 77%. As with recall, we compute both overall precision, as well as the precision for each category/species label.

3) F-Measure: The F-measure, F_1, is the *harmonic mean* of the precision, P and the recall, R:

$$F_1 = \frac{2}{\frac{1}{P} + \frac{1}{R}} = 2 \cdot \frac{P \cdot R}{P + R}$$

While we prefer maximizing this value, a value near 0.5 means we are doing well.

B. Adjusting k

Adjusting k is an important first step. We investigate k values ranging from 1 to 17, but focus primarily on $k \leq 12$. At this point, we do not filter the results in order to focus primarily on the affect of the size of the k-nearest neighbors list. Thus, α is 0, allowing for the full k list to factor into classification.

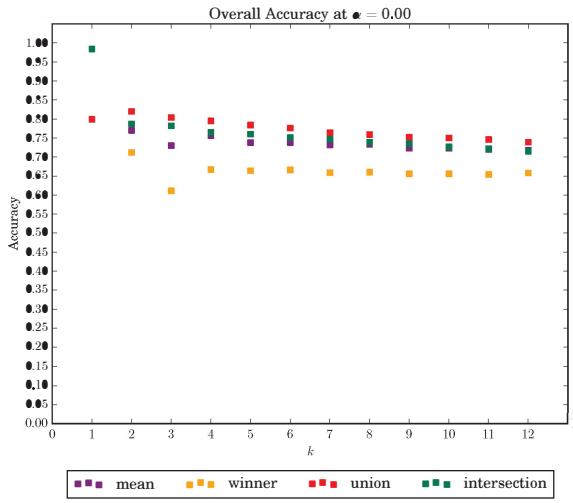

Fig. 2. The accuracy of all classifications performed with CPLOP across the four different algorithms with $\alpha = 0.00$ shows little improvement for $k > 5$. We look at only the percentage of correct classifications, since that value is equivalent to the precision and the recall.

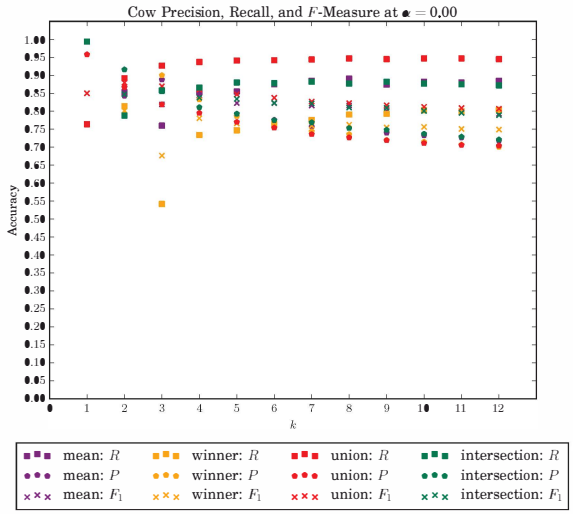

Fig. 3. There are 1838 Cow isolates in CPLOP. For most resolution algorithms, we observe little improvement when $k > 5$.

Overall, for $k \geq 5$, the accuracy does not improve, but instead levels off. Depending on the resolution algorithm, this value is between 65% and 75% accuracy, as shown in Figure 2. By "overall," we mean that for every classification, we validated if it was correct and calculated what proportion to all classifications made that represents to determine accuracy. When looking at all classifications, precision and recall are identical values, as is F-measure.

One good example is the Cow. As Figure 3 shows, Cow follows a trend similar to the overall accuracy, staying roughly between 70% and 95% accurate. Certain algorithms get worse for $k > 5$, while other improve.

Figure 4 examines the relationship between R and P. This can help us understand the trade offs of choosing one k over another. We will later build a meaningful strategy for how confident we are at recalling a species versus our confidence

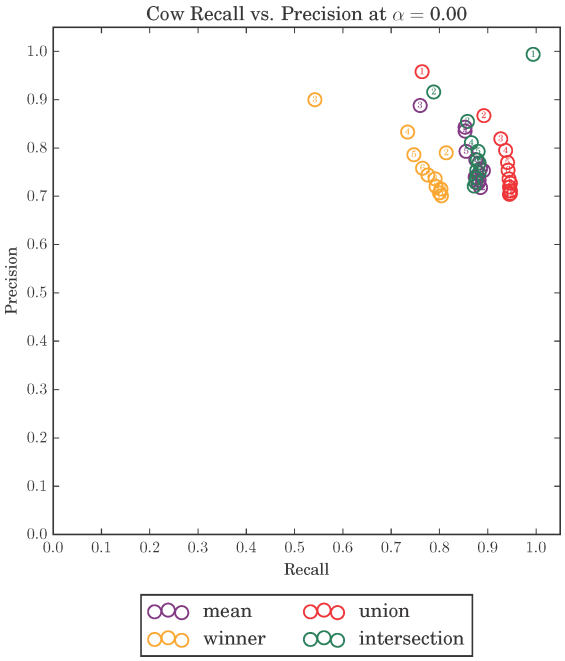

Fig. 4. There are 1838 Cow isolates in CPLOP. Looking at the Recall as it compares to the Precision for $\alpha = 0.99$ allows us to visualize the tradeoffs we make when picking a k value. Labeled within each datapoint is the k value at that point

in a classification of a species.

C. Adjusting α

By adding a threshold value, we investigated whether this further limitation improves the accuracy by restricting outliers from populating a k-nearest neighbors list. We investigate $\alpha = \{0.00, 0.98, 0.99\}$. Outside of this study, $\alpha = 0.99$ defines the boundary between strains. One reason we investigate 0.98 is to see whether loosening our definition of strain differentiation gives us a better accuracy.

Overall, we observe that the accuracy slightly improves as we increase the α threshold. Figure 5 shows that overall, the accuracy increases as we increase α.

Adding the α made minimal changes to the accuracy of Cow classifications, so only the recall versus precision is shown in Figure 6. More details into how α affect the classification accuracy can be seen in Tables II, III, and IV.

D. Adjusting the Algorithm

Choosing which algorithm to resolve the two different regions of each isolate is an important step. We investigate the differences between the aforementioned four algorithms as they relate to k and α values and how each differ among species of different representation. With library-based-MST, it is important to realize the representation of a species in the library may heavily skew the accuracy of the library.

While interpreting the data, we state that there may be some "%" increase or decrease which we intend to mean the increase in the raw value of the percentage. Additionally, values in the tables represent the proportion of the three metrics, but

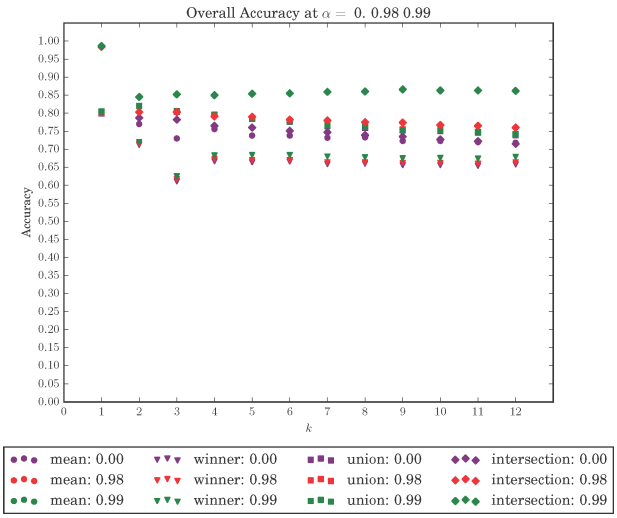

Fig. 5. Shown is the accuracy of all classifications performed with CPLOP across the four different algorithms. We find that the accuracy of certain resolution algorithms perform better with higher α values.

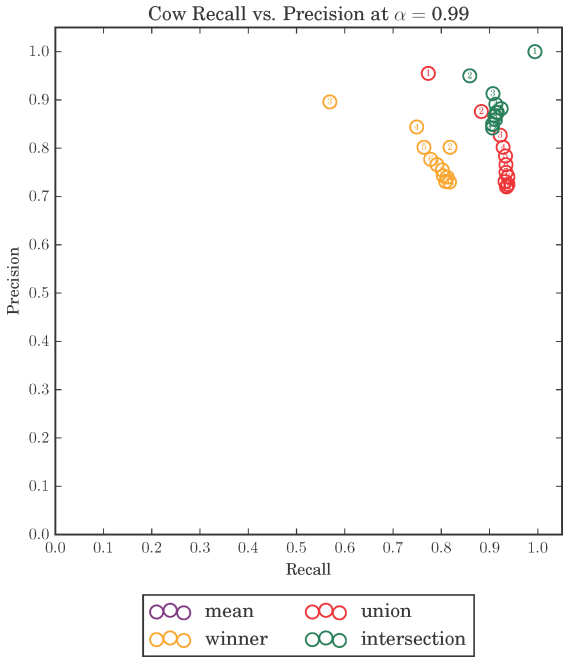

Fig. 6. There are 1838 Cow isolates in CPLOP. Increasing the α for a species with this many isolates made minimal improvements to the accuracy on all but the resolution by intersection algorithm, which, when compared to Figure 4 noticeably improved.

are easily interpreted as percentages. Section IV-A explains the meaning of each precision (P), recall (R), and F-measure (F_1).

Overall, with $\alpha = 0.00$, Figure 2 illustrates that the resolution by union algorithm consistently performs better. For $k = 7$ and $\alpha = 0.00$, Table II shows that using the resolution by unions algorithm performs with 76.4% accuracy with meanwise and resolution by winner and intersection respectively achieving 73.2%, 65.9%, and 74.7 accuracy%.

TABLE II. PRECISION (P), RECALL (R), AND F-MEASURE (F_1) OVERALL AND FOR PARTICULAR SPECIES AT k=7, $\alpha = 0.00$.

		Meanwise			Winner		
Species	|Isolates|	P	R	F_1	P	R	F_1
Overall	4682	0.732	0.732	0.732	0.659	0.659	0.659
Human	1471	0.857	0.922	0.888	0.771	0.861	0.814
Cow	1718	0.757	0.885	0.816	0.744	0.776	0.760
Pigeon	194	0.420	0.242	0.307	0.280	0.253	0.266
Dog	149	0.596	0.436	0.504	0.449	0.356	0.397
Wild Turkey	72	0.383	0.250	0.303	0.277	0.181	0.219
Chicken	40	0.182	0.050	0.078	0.143	0.075	0.098
Cat	39	0.571	0.308	0.400	0.438	0.359	0.395
Bat	37	0.857	0.973	0.911	0.692	0.973	0.809
Seagull	11	0.000	0.000	0.000	0.000	0.000	0.000

		Union			Intersection		
Species	|Isolates|	P	R	F_1	P	R	F_1
Overall	4682	0.764	0.764	0.764	0.747	0.747	0.747
Human	1471	0.843	0.930	0.884	0.839	0.925	0.880
Cow	1718	0.736	0.944	0.827	0.769	0.882	0.822
Pigeon	194	0.569	0.170	0.262	0.470	0.284	0.354
Dog	149	0.761	0.450	0.566	0.649	0.497	0.563
Wild Turkey	72	0.688	0.306	0.424	0.510	0.361	0.423
Chicken	40	0.000	0.000	0.000	0.250	0.100	0.143
Cat	39	0.889	0.410	0.561	0.571	0.308	0.400
Bat	37	0.857	0.973	0.911	0.857	0.973	0.911
Seagull	11		0.000			0.000	

TABLE III. PRECISION (P), RECALL (R), AND F-MEASURE (F_1) OVERALL AND FOR PARTICULAR SPECIES AT k=7, $\alpha = 0.98$.

		Winner		
Species	|Isolates|	P	R	F_1
Overall	4682	0.664	0.664	0.664
Human	1471	0.773	0.865	0.816
Cow	1718	0.749	0.777	0.763
Pigeon	194	0.287	0.254	0.269
Dog	149	0.448	0.349	0.392
Wild Turkey	72	0.308	0.222	0.258
Chicken	40	0.150	0.075	0.100
Cat	39	0.467	0.359	0.406
Bat	37	0.692	0.973	0.809
Seagull	11	0.000	0.000	0.000

		Union			Intersection		
Species	|Isolates|	P	R	F_1	P	R	F_1
Overall	4682	0.767	0.767	0.767	0.780	0.780	0.780
Human	1471	0.845	0.930	0.885	0.876	0.950	0.912
Cow	1718	0.742	0.943	0.831	0.799	0.894	0.844
Pigeon	194	0.538	0.181	0.271	0.521	0.333	0.406
Dog	149	0.756	0.456	0.569	0.698	0.536	0.606
Wild Turkey	72	0.697	0.319	0.438	0.571	0.387	0.461
Chicken	40	0.000	0.000	0.000	0.308	0.121	0.174
Cat	39	0.889	0.410	0.561	0.632	0.353	0.453
Bat	37	0.857	0.973	0.911	0.878	0.973	0.923
Seagull	11		0.000		0.000	0.000	0.000

TABLE IV. PRECISION (P), RECALL (R), AND F-MEASURE (F_1) OVERALL AND FOR PARTICULAR SPECIES AT k=7, $\alpha = 0.99$.

		Winner		
Species	|Isolates|	P	R	F_1
Overall	4682	0.680	0.680	0.680
Human	1471	0.780	0.872	0.823
Cow	1718	0.766	0.791	0.778
Pigeon	194	0.314	0.263	0.286
Dog	149	0.527	0.401	0.455
Wild Turkey	72	0.320	0.222	0.262
Chicken	40	0.167	0.100	0.125
Cat	39	0.433	0.333	0.376
Bat	37	0.720	0.973	0.828
Seagull	11	0.429	0.273	0.334

		Union			Intersection		
Species	|Isolates|	P	R	F_1	P	R	F_1
Overall	4682	0.766	0.766	0.766	0.859	0.859	0.859
Human	1471	0.843	0.925	0.882	0.926	0.979	0.952
Cow	1718	0.750	0.934	0.832	0.874	0.914	0.894
Pigeon	194	0.476	0.205	0.287	0.611	0.468	0.530
Dog	149	0.739	0.456	0.564	0.838	0.738	0.785
Wild Turkey	72	0.719	0.319	0.442	0.667	0.455	0.541
Chicken	40	0.000	0.000	0.000	0.000	0.000	0.000
Cat	39	0.938	0.385	0.546	0.909	0.588	0.714
Bat	37	0.837	0.973	0.900	0.973	1.000	0.986
Seagull	11	0.000	0.000	0.000		0.000	

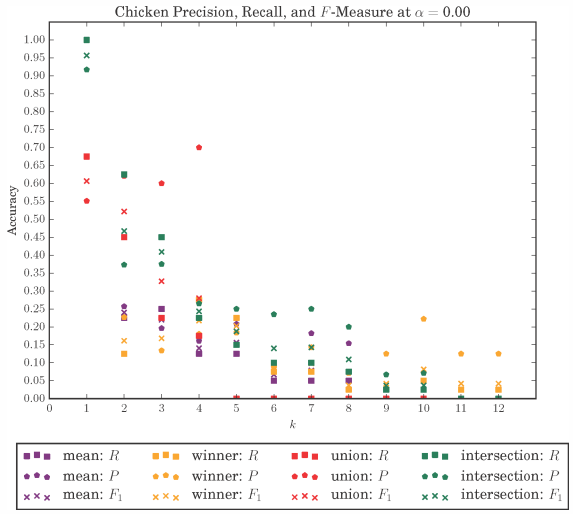

Fig. 7. There are 40 Chicken isolates in CPLOP. Unfortunately, due to their low representation in CPLOP, classification accuracy is low.

Poorly represented species, like the Cat, Chicken, and Seagull did not benefit from the resolution by union algorithm, each achieving no classifications, correct or otherwise.

Once we restrict with a somewhat loose threshold of 0.98, overall we see that the intersection method provides the best accuracy, improving on non-thresholded values. For $k = 7$ and $\alpha = 0.98$, the intersection algorithm achieves 78.0% accuracy%, while resolution by winner and union respectively achieve 66.4% and 76.7% accuracy.

Table III shows that a handful of poorly represented species achieved slightly better results when $\alpha = 0.98$. Notably, the intersection algorithm F-measure increased slightly for Wild Turkey, Cat, and Chicken on the order of 3%.

Unfortunately, the meanwise algorithm fails to classify when we use a large enough α and thus we have ommited the results in Tables III and IV. In certain cells of the tables, including Table II, empty values in either P or F_1 mean no classifications were made of that species.

Restricting with $\alpha = 0.99$, our definition of strain differentiation, overall accuracy improves more with resolution by intersection and less so with resolutions by winner and union, garnering 85.9%, 68.0%, and 76.6% accuracy respectively. Again, meanwise resolution fails to produce any classifications.

For poorly represented species, we see some similar improvements for P, R, and F_1, but also some exceptions. Wild Turkey for example, improves by about 2%-3% for resolutions by winner and union and 11% for resolution by intersection, while Cat decreases by 3% for resolution by winner, but improves by 6% and 47% for resolution by union and intersection.

E. Poorly Represented Species

Some species had worse accuracy than the overall accuracy. In particular, species such as Chicken with only 40 isolates representing it showed similar leveling of accuracy for $k > 5$, but had far poorer accuracy, as shown in Figure 7. For $k > 5$, the accuracy of classifying chicken ranges from as low as 10% to a peak of 26%. The classification accuracy for many species in CPLOP heavily relies on its representation in CPLOP.

One notable exception is the Bat. In everyone application of our k-Nearest Neighbors algorithms, Bat has above 95% accuracy. It is possible that due to their small size and relative dietary segregation from the surrounding environment that the strains of *E. coli* stay particularly unique. It may also be a quirk of the fact that each isolate comes from a single host, making it difficult to draw conclusions from such results.

V. Discussion & Conclusion

Generally, when using k-Nearest Neighbors, it is preferred to use single digit k values. Through our investigation of these various k-Nearest Neighbors classification algorithms, we find that that general advice holds true. For our dataset, using $k \geq 5$ does not produce much different results. Choosing $k < 5$ is a dangerous notion, since it is likely that an outlier may make its way into the k-nearest neighbors list, confounding the results. Staying with $5 \leq k \leq 9$ appears to be a safe and reasonable option, providing a good balance between accuracy and filtering of isolates.

Outside of this study, we choose to differentiate between strains of *E. coli* using $\alpha = 0.99$. It appears that using this value is advantageous. There were, however, some exceptions to those results, motivating us to consider non-thresholded k-nearest neighbors lists when classifying an unknown isolate.

The four resolution algorithms — meanwise, winner, union, and intersection — each have their own quirks and behaviors as we alter k and α.

Meanwise, which currently uses the Euclidean norm to resolve different metrics, did not respond to the α threshold and completely stopped classifying anything for α near 1. This is very likely due to Euclidean norm mapping $([0,1],\ldots,[0,1]) \rightarrow [0,\sqrt{1+\cdots+1}]$. To get around this, we multiplied the resulting norm by a factor of $\sqrt{2}$, which may have unexpected results. We may investigate this further, or choose a more natural norming method, like arithmetic or geometric mean. With no α filtering, it performed third best with an overall 73.2% classification accuracy.

Winner performs worst, classifying accurately between 65% and 68% of the time. Some alterations to this algorithm may make it more reliable, such as only counting the species that appear in all lists.

Unionwise performs very well. Without filtering the k-nearest neighbors lists by α, we find that the unionwise method classifies best, with an overall accuracy of 76.4%. However, once we add in α filtering, the unionwise does not improve, staying relatively close to 76%.

Intersection performs best when we use α. This is likely due to the "list" actually being a set of common isolates. Overall, without filtering, the accuracy was 74.7%, 78%, and 85.9% for α =0.00, 0.98, and 0.99 respectively.

Overall, we find that the intersection algorithm performs the best and recommend moving forward with it. While unionwise did perform well, it did not respond well to thresholding and still did not perform as well as the intersection algorithm overall. Meanwise and winner may be more useful with previously mentioned modifications and we may investigate these in the future.

Poorly distributed representation of species and environmental incomparabilities are issues endemic to library-based MST. CPLOP has an overabundance of Cow and Human isolates, and an underrepresentation of many of the species in the database. This dilutes the k-nearest neighbors list considerably for species like the Chicken and Cat.

Library population issues aside, environmental limitations are another concern for accuracy. Nearly every sample in the library comes from a 30 mile radius around Cal Poly, making the collected pyroprints potentially incomparable to pyroprints collected from a different region.

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