Advanced Statistics: Application of supervised and unsupervised methods to biological data

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2025-04-27

Abstract: Biological data with twenty features and four categorical class labels is explored and analysed using advanced statistical techniques in this report. Both supervised and unsupervised methods were implemented and evaluated, and the broad selection of models includes logistic regression, support vector machine, random forest, agglomerative hierarchical clustering, and gaussian mixture modelling. Comparing the results achieved using a selection of models with different underlying principles gives insight into the nature of the data. For example, the success of model-based clustering compared to hierarchical clustering and tree-based learning suggests the lack of hierarchy among the categorical labels, and the success of factor analysis as a dimensionality reduction technique suggests the presence of underlying biological mechanisms leading to several of the features arising together. Models achieving over 90% accuracy were produced, but all models performed notably worse at separating one of the categories that overlapped the other three.

Contents

List of Figures

List of Tables

1 Introduction

This report takes a moderately sized biological dataset containing 3000 observations, where each observation having 20 numeric varables and one catagorical label. These data are explored thoroughly before being used to train and test an array of statistical modelling techniques.

This is an interesting project because the origin and meaning of the variables in the data are completely unknown - an unusual scenario in the data science field, where usually it is the domain knowledge and problem context that inform the selection and implementation of statistical methods. Here, with this relationship reversed, algorithms have been chosen so that the evaluation of their performance can attempt to uncoverthe underlying biological significance of the variables.

Achieving meaningful results in this task shows the importance of supervised and unsupervised learning to this field, where classification algorithms can build valuable models that have high impact on society such as disease diagnosis models, and unsupervised learning techniques can create breakthoughs in identifying clusters of data that lead to new discoveries and classifications (Cai et al. 2017).

2 Methods

2.1 Data Description

Each of the 3000 observations has 20 numeric features and a label placing it in one of four catagorical classes. Though exploritory data analysis, two groups of correlated features were identified. Outliers were identified and removed using z score method. One feature transformed using logarithm to create a more normal distribution to improve the performance of models. All features were scaled and centered. After the preprocessing of the data, 2776 usable observations remained ("(PDF) The Power of Outliers (and Why Researchers Should Always Check for Them)" n.d.).

Bootstrap sampling was used to create a larger dataset so that the performance of the models could be compared between the original and bootstrapped data.

Table 1: Feature Descriptions

Variable Name	No. missing values	mean	Std deviation	min	25th %ile	median	75th %ile	max
X1	2	9.876	0.764	6.840	9.356	9.872	10.399	12.355
X2	0	10.151	1.040	6.538	9.445	10.138	10.855	14.021
X3	1	8.861	0.871	6.424	8.243	8.847	9.445	12.216
X4	2	8.939	1.275	3.875	8.088	8.926	9.805	13.351
X5	0	13.853	0.942	10.527	13.236	13.858	14.492	16.557
X6	0	8.151	1.026	4.815	7.447	8.134	8.856	11.871
X7	1	0.426	0.278	0.000	0.185	0.375	0.678	1.301
X8	3	0.234	0.197	0.000	0.102	0.170	0.300	1.230
X9	0	0.717	0.247	0.006	0.532	0.751	0.888	1.679
X10	0	0.378	0.155	0.000	0.281	0.372	0.469	1.199
X11	1	9.175	1.087	6.031	8.412	9.077	9.860	13.027
X12	0	11.930	0.977	8.046	11.290	11.913	12.594	15.478
X13	1	8.228	0.806	4.919	7.719	8.244	8.746	11.226
X14	2	7.846	1.238	3.574	7.022	7.884	8.689	12.413
X15	1	10.701	0.962	7.572	10.054	10.701	11.344	14.037
X16	1	7.814	1.052	3.801	7.132	7.830	8.521	11.668
X17	2	0.504	0.221	0.001	0.348	0.502	0.653	1.315
X18	3	0.682	0.204	0.004	0.544	0.686	0.819	1.390
X19	0	0.544	0.254	0.000	0.363	0.545	0.710	1.518
X20	0	0.589	0.231	0.012	0.434	0.587	0.746	1.353

This description of the predictive features shows the range of scales, ranging by and order of magnitude (Table 1). Several models such as support vector machine analysis are affected by the scale and centering of the data it learns from, so it this was identified as an important preprocessing step that had to be performed.

2.2 Exploratory Data Analysis Approach

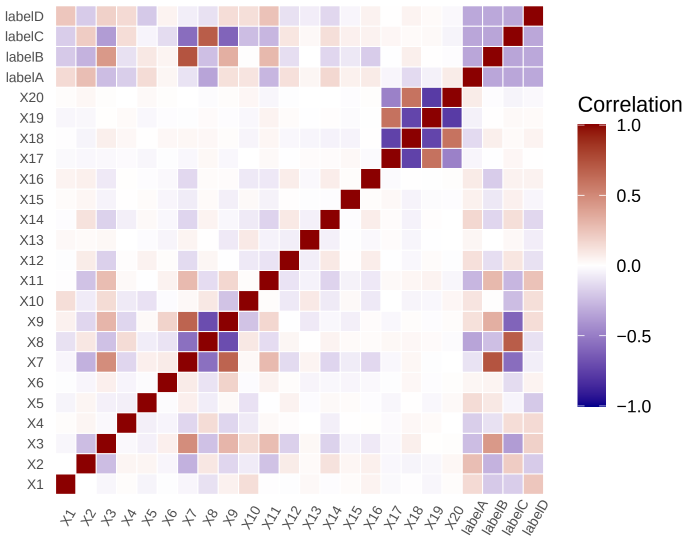

Figure 1: **Feature and Class Correlation Matrix:** *highlighting relationships between variables and relationships with catagorical labels*

Within the data there are two groups of features that correlate together - X7, X8, and X9, and X17 to X20 (Figure 1). Noticing groups of correlated features is important since some models such as logistic regression and SVM will struggle with multicolinearity. This will lead us to attempt feature selection or dimensionality reduction with these models, or choose alternative algorithms that are more robust in these cases such as tree-based algorithms.

The difference between these two group is that while X7, X8, and X9 are three features with some of the strongest correlations with the label values, all of X17 to X20 are features without significant correlations. This would lead us to believe that X17 - X20 have low predictive power in classification that aim to predict the class label and so removing them entirely would be a justifyable approach.

Among the other columns, we see that there are definitly some columns with stronger correlations than others.

To produce the correlation matrix, the four catagorical labels were one hot encoded to create four binary columns. This allows us to see that several features have strong predictive power for one or more label but not all. For example, X8 has high correlations with classes A, B, and C, but none very low correlation with class D. This contrasts with a feature like X11 which has equal magnitude of correlation across all four labels.

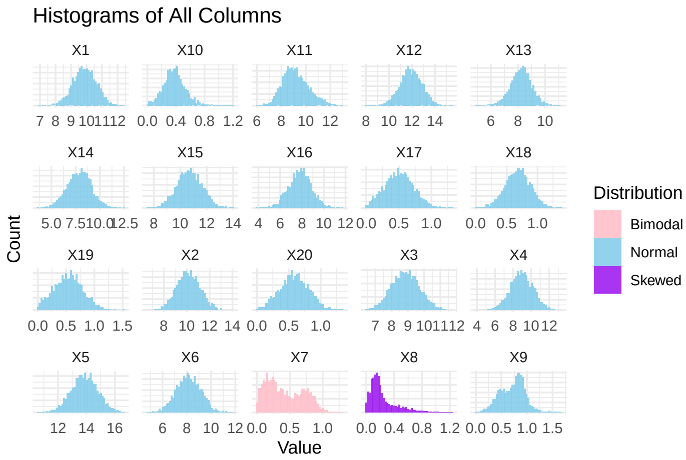

Figure 2: **Distributions within feature columns:** *histograms showing scale and skewness of data*

The majority of the 20 predictive variables followed normal distributions. Noteable exceptions were X8, which is heavily right skewed, and X7 which has a bimodal appearance (Figure 2). With both of these features showing strong correlations with the labels leading to a high probability that they have strong predictive power, they should not be removed. X8 will be transformed, and the natural logarithm of X8 will be used in all modelling.

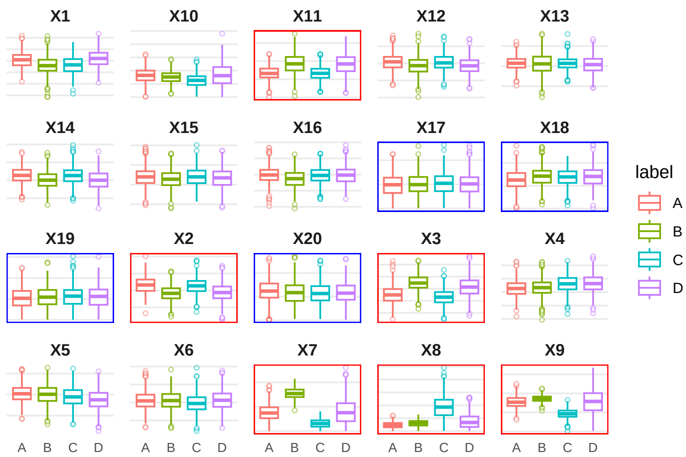

Figure 3: **Distributions within feature columns:** *Boxplots by class label by feature*

Across the entire dataset, the distribution of labels is uniform, with roughly on quarter of the observations falling into each category.

When the distribution of each feature is observed by class label some features have significantly different characteristics for each class. In Figure 3, highlighted with a red boarder are features where we can see notable differences in the key descriptive statistics such as medians and interquartile ranges between different classes. Highlighted in blue boarders are the features where the boxplots look almost identical from one feature to another. This gives insight into which features will be important for building effective models - it is expected that features such as X7 with very different stats per class will be useful at creating decision boundaries or defining distributions for model-based clustering.

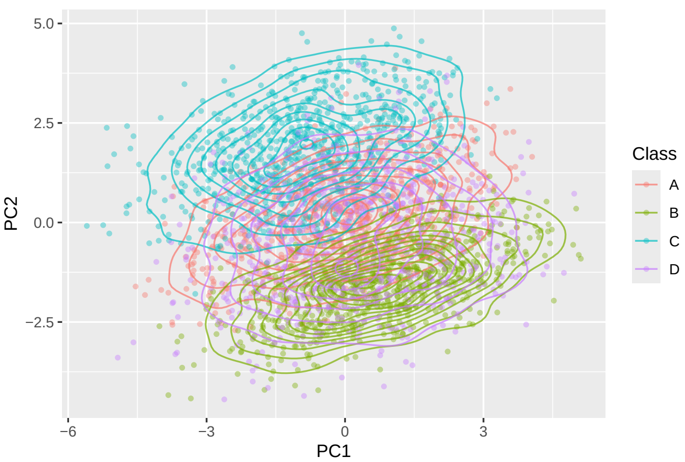

Figure 4: **PCA plotting of class labels:** *scatterplot showing clustering tendency of catagorical classes*

It is important to learn about the structure of the underlying data in order to understand the chances of sucess with modelling methods. Using Principle Componant Analysis captures most of the information in the system in one scatter plot (Figure 4). This shows that there is some underlying structure to the data that will lead to the formation of clusters, although it is fairly loose in this plot. Annotating the points with the target label shows us that classes A, B, and C form elipsoidal groups that are roughly equal in size and orientation, and offset along the second principle component. The fourth class (D) forms a larger elipsoid that overlaps significantly with the other three.

The seperation of the first three classes suggests there is information in the features that allow statistical models to decern between them, but the overlap of class D means that this class may have more misclassifications. It may be challenging to find an approach that is effective for this label.

The fact that the three classes that are distinct are displaced along the second principle componant and not the first principle component means that it is the dimensions with less variation that distinguish them. It would be easier to seperate the groups with predictive models if they were offset along the first principle conmponent.

To learn more about the underlying structure of the dataset, the clustering tendency can be examined by calculating the Hopkins statistic, where a score close to 1 indicates strong clustering tenency, a score of 0.5 indicates random distribution of occurences, and 0 a uniform distribution (Wright 2022).

Table 2: Hopkins Statistic Scores

Columns used	Hopkins Statistic Score
All Features + Label	0.9999815
All Features with Label Removed	0.9999718
All Features Binary Class D vs Rest	0.9999749
Features X2,X3,X7,X8,X9,X11	0.9964180
Features X17,X18,X19,X20	0.9983264
Features X1,X4,X6,X10,X12,X13,X14,X15,X16	0.9910779

There is a very high clustering tendency for various treatments of the data (Table 2). The statistic remains high when the label is removed from the feature set. This is promising for pursuing unsupervised approaches, since it confirms the 20 numeric features contain the information that are structuring the data into clusters, rather than the label itself providing a significant amount of this structure. If the score dropped when the label was removed, this would suggest that the label was necessary for dividing the data into classes and the features themselves did not contain sufficient predictive information to do so.

Similarly, the score remains high for three different groups of features. These three groups are the groups we see with different coloured boarders in the boxplots. This means that even the features with low correlation with the labels provide structure to the data. This could suggest that there are other ways that the data could be structured when using unsupervised models that do not correspond with the given labels.

Overall, we see from exploritory data analysis that this data contains high amounts of information usable for predictive modelling, and a high clustering tendency which is promising for unsupervised clustering. Multicolinarity has been observed among several features that could hinder model performance.

2.3 Supervised Learning Methods

2.3.1 logistic regression - including class weighting, L2 regularization, and feature selection.

A simple model, logistic regression is quick to implement and will reveal more about the data, in particular how different features contribute to predictions ("Multinomial Logistic Regression in R" 18:24:37+00:00).

There are variations such as weighting and regularization (James et al. 2023, 240–53) which will be implemented - the success of lack of success of these techniques will reveal characteristics about our data that will be valuable to inform the selection and implementation of other models ("Weighted Logistic Regression in R" 09:33:43+00:00)

2.3.2 Random forest, including feature selection and tuning of mtry.

Random Forest was chosen due to its resilience. One of the more robust option, it is a good choice for handling the data without extra processing. Several characteristics of the data have been identified that may cause othe models such as logistic regression and SVM to struggle: - Correlated of features - Features that appeared to have low linear correlations with the label values (from heatmap in eda) but still contributed to the predictive ability of the model. This suggests there might be some non-linear relationships between features and the target variable - Features that aren't perfectly normally distributed, such as the bimodal peak in X7

Random Forest is a robust algorithm with few underlying assumptions that will handle these considerations well (James et al. 2023, 346–47). Random Forest resists overfitting because of the sampling approach, it handles non linearity well, and it is naturally suited to multinomial classifications problems, like the one we have with four possible values for label. I also think that tree based models may peform well at distinguishing class A from class D, which was the biggest challenge that held back our logistic regression modelling. This is because it can prioritize at an early node in the tree a feature such as X11, which is one of the few that had high importance for descerning between class A and D, and then refine the selection in further nodes.

2.3.3 SVM, including feature selection, tuning of kernel selection, gamma, and cost parameters.

SVM is a powerful and popular algorith. SVM has options for different kernals that can be tuned, and this is a promising approach to solving the challenges of seperating class A from class D that is evedent from the data analysis and the results of logistic regression (James et al. 2023, 378 - 382). It might be the case that A and D aren't linearly seperable, but a non-linear kernal will have success.

2.4 Unsupervised Learning Methods

2.4.1 Agglomerative hierarchical clustering, including tuning of linking metric.

Agglomerative heirarchical clustering was chosen because it is interesting to explore a model where the number of clusters is not specified and let the natural structure of the data reveal itself.

Biological data is often naturally heirarchical, for example animals can be classified by deviding them into first kingdoms, then families of species, and finally species and sub-species (Cai et al. 2017).

Although the exact meaning of each feature in the data isn't known, it is biological in origin (perhaps gene expressions or environmental factors). This means that there may be a heirarchy of classes in our data.

Using this method without specifying that there are four values for label might reveal that some of the labels have a strong tendency to form sub classes, or that there is little structure in the data to justify asserting there are four classes. These would both be interesting finds.

It is also a model that handles the feature correlation well, which allows us to keep in all the collumns that correlate like X7, X8, and X9.

2.4.2 Gaussian mixture model clustering, including model seclection, regularization and dimensionality reduction using factor analysis.

So far while working with this data set, it has been challenging to seperate class D. By plotting the results of some of the methods in specific dimensions, we have been able to show that class D significantly overlaps the other classes. We have also seen from the two dimensional PCA scatterplot that this is general overlap between all the classes in the the first two principle componants of the data.

Lots of clustering methods struggle with seperating overlapping clusters, so for the final method I wanted to choose one that might perform better with this challenge in mind. I have chosen to try a gaussian mixture model (gmm) - a model-based clustering technique that assumes that all the data is distributed according to the combination of different normal distributions("Gaussian Mixture Model Explained" n.d.). There is a fair chance of gmm performing well on our dataset because it is probabalistic, calculating the probability that a data point is in each cluster. This can help it perform better than other methods like K-means when there aren't clear boundaries between the clusters such as we see with class D.

Another advantage it has is that it has some flexibility in the geometry of the clusters it produces, unlike k-means which tends to produce spherical clusters(Géron 2023). This is important because we have seen that in some dimensions our classes produce ellipsoidal clusters. It also operates on very different fundemental principles to our other unsupervised method - agglomerative hierarchical clustering - so it will be good to compare the two. If model-based clustering performs much better then it could suggest that the classes of our data are not heirarchical in nature.

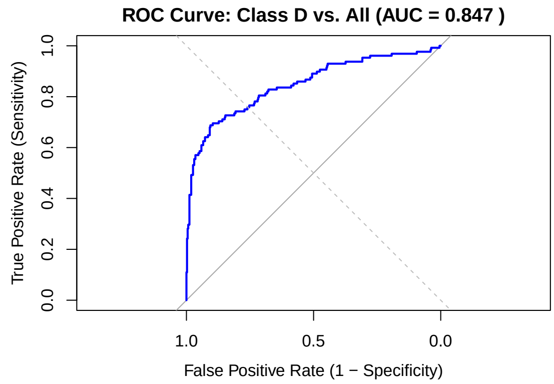

Figure 5: **Simple Logistic Regression ROC Plot:** *ROC plot for Class D vs Not Class D*

3 Results

3.1 Supervised Learning Results

In this section the results of various models are presented through performance metrics such as accuracy, recall, precicion and f1 score (Grus 2019). More details on the approach to tuning and evalutation can be found in notebooks (Hill, n.d.)

3.1.1 Logistic Regression

Table 3: Simple Logistic Regression Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	128	1	3	14
B	0	129	0	20
C	0	0	140	17
D	12	8	4	77

Table 4: Simple Logistic Regression Overall Statistics

Statistic	Value
Accuracy	0.8571
Kappa	0.8091
AccuracyLower	0.8252
AccuracyUpper	0.8852
AccuracyNull	0.2658
AccuracyPValue	0.0000

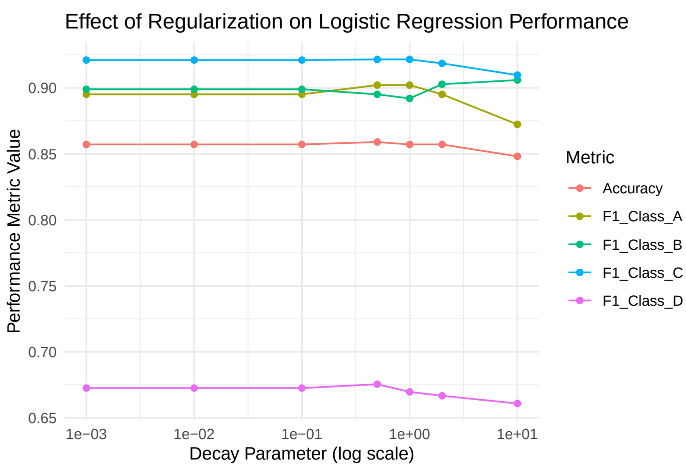

Figure 6: **Regularized Logistic Regression**

Table 5: Simple Logistic Regression Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9143	0.9348	0.9524	0.6016
Specificity	0.9564	0.9518	0.9581	0.9435
Pos Pred Value	0.8767	0.8658	0.8917	0.7624
Neg Pred Value	0.9705	0.9777	0.9823	0.8872
Precision	0.8767	0.8658	0.8917	0.7624
Recall	0.9143	0.9348	0.9524	0.6016
F1	0.8951	0.8990	0.9211	0.6725
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2315	0.2333	0.2532	0.1392
Detection Prevalence	0.2640	0.2694	0.2839	0.1826
Balanced Accuracy	0.9354	0.9433	0.9553	0.7725

Table 6: Simple Logistic Regression with selected features (X17,X19,X20 removed) Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	122	1	4	16
B	0	128	0	18
C	1	0	141	19
D	17	9	2	75

Table 7: Simple Logistic Regression with selected features (X17,X19,X20 removed) Overall Statistics

Statistic	Value
Accuracy	0.8427
Kappa	0.7897
AccuracyLower	0.8096
AccuracyUpper	0.8720
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 8: Simple Logistic Regression with selected features (X17,X19,X20 removed) Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.8714	0.9275	0.9592	0.5859
Specificity	0.9492	0.9566	0.9507	0.9341
Pos Pred Value	0.8531	0.8767	0.8758	0.7282
Neg Pred Value	0.9561	0.9754	0.9847	0.8822
Precision	0.8531	0.8767	0.8758	0.7282
Recall	0.8714	0.9275	0.9592	0.5859
F1	0.8622	0.9014	0.9156	0.6494
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2206	0.2315	0.2550	0.1356
Detection Prevalence	0.2586	0.2640	0.2911	0.1863
Balanced Accuracy	0.9103	0.9421	0.9550	0.7600

The results from logistic regression were promising given the simplicity of the model, with an accuracy

Table 9: Simple Logistic Regression with selected features (X8,X9 removed) Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	112	3	8	16
B	4	119	0	14
C	14	0	127	28
D	10	16	12	70

Table 10: Simple Logistic Regression with selected features (X8,X9 removed) Overall Statistics

Statistic	Value
Accuracy	0.7740
Kappa	0.6978
AccuracyLower	0.7368
AccuracyUpper	0.8082
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 11: Simple Logistic Regression with selected features (X8,X9 removed) Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.8000	0.8623	0.8639	0.5469
Specificity	0.9346	0.9566	0.8966	0.9106
Pos Pred Value	0.8058	0.8686	0.7515	0.6481
Neg Pred Value	0.9324	0.9543	0.9479	0.8697
Precision	0.8058	0.8686	0.7515	0.6481
Recall	0.8000	0.8623	0.8639	0.5469
F1	0.8029	0.8655	0.8038	0.5932
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2025	0.2152	0.2297	0.1266
Detection Prevalence	0.2514	0.2477	0.3056	0.1953
Balanced Accuracy	0.8673	0.9095	0.8802	0.7287

Table 12: Weighted Logistic Regression Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	128	1	3	13
B	0	128	0	19
C	0	0	139	16
D	12	9	5	80

of 85% (. weighting did nothing, as expected. regularization didn't really do anything. feature selection did not improve the model. We saw that the model particularly underperformed at classifying class D correctly.

3.1.2 Random Forest

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-0.04366812 0.01
```

Table 13: Weighted Logistic Regression Overall Statistics

Statistic	Value
Accuracy	0.8571
Kappa	0.8091
AccuracyLower	0.8252
AccuracyUpper	0.8852
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 14: Weighted Logistic Regression Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9143	0.9275	0.9456	0.6250
Specificity	0.9588	0.9542	0.9606	0.9388
Pos Pred Value	0.8828	0.8707	0.8968	0.7547
Neg Pred Value	0.9706	0.9754	0.9799	0.8926
Precision	0.8828	0.8707	0.8968	0.7547
Recall	0.9143	0.9275	0.9456	0.6250
F1	0.8982	0.8982	0.9205	0.6838
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2315	0.2315	0.2514	0.1447
Detection Prevalence	0.2622	0.2658	0.2803	0.1917
Balanced Accuracy	0.9366	0.9409	0.9531	0.7819

Table 15: Logistic Regression Performance with Different Regularization Parameters

Decay	Accuracy	Kappa	F1 Score (A)	F1 Score (B)	F1 Score (C)	F1 Score (D)
0.001	0.8571	0.8091	0.8951	0.8990	0.9211	0.6725
0.01	0.8571	0.8091	0.8951	0.8990	0.9211	0.6725
0.1	0.8571	0.8091	0.8951	0.8990	0.9211	0.6725
0.5	0.8590	0.8115	0.9021	0.8951	0.9216	0.6754
1	0.8571	0.8090	0.9021	0.8920	0.9216	0.6696
2	0.8571	0.8090	0.8951	0.9028	0.9186	0.6667
10	0.8481	0.7969	0.8723	0.9059	0.9097	0.6608

Table 16: Confusion Matrix for Best Regularized Logistic Regression Model (Decay = 0.5)

	Reference			
Prediction	A	B	C	D
A	129	1	3	13
B	0	128	0	20
C	0	0	141	18
D	11	9	3	77

Table 17: Basic Random Forest Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	131	1	1	17
B	1	135	0	9
C	1	0	144	12
D	7	2	2	90

```
-0.004366812 0.01
```

Table 18: Basic Random Forest Overall Statistics

Statistic	Value
Accuracy	0.9042
Kappa	0.8719
AccuracyLower	0.8765
AccuracyUpper	0.9274
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 19: Basic Random Forest Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9357	0.9783	0.9796	0.7031
Specificity	0.9540	0.9759	0.9680	0.9741
Pos Pred Value	0.8733	0.9310	0.9172	0.8911
Neg Pred Value	0.9777	0.9926	0.9924	0.9159
Precision	0.8733	0.9310	0.9172	0.8911
Recall	0.9357	0.9783	0.9796	0.7031
F1	0.9034	0.9541	0.9474	0.7860
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2369	0.2441	0.2604	0.1627
Detection Prevalence	0.2712	0.2622	0.2839	0.1826
Balanced Accuracy	0.9449	0.9771	0.9738	0.8386

Table 20: Basic Random Forest Feature Importance

	A	B	C	D	MeanDecreaseAccuracy	MeanDecreaseGini
X7	56.558	118.667	49.213	12.349	95.167	359.608
X8	65.991	25.980	62.515	-6.441	69.507	251.828
X10	46.550	24.751	52.066	21.225	60.814	132.552
X9	36.974	30.870	52.227	14.993	57.843	241.034
X11	41.356	11.814	39.916	29.586	52.587	113.984
X3	27.784	27.346	32.775	14.129	44.176	108.661
X2	19.998	13.586	13.090	8.573	25.746	62.498
X1	8.705	13.112	11.541	15.451	22.705	54.390
X4	14.772	4.246	2.835	7.697	16.201	40.565
X5	13.007	3.643	0.538	12.547	15.756	38.826
X14	10.934	1.717	7.500	5.310	11.994	36.633
X13	6.940	-0.114	6.335	7.238	10.488	30.080
X18	10.786	4.458	3.321	0.596	9.840	27.809
X12	4.842	-0.140	5.828	5.614	8.871	28.373
X20	6.059	3.517	3.691	-0.660	6.853	21.973
X19	3.013	4.775	1.034	-0.136	4.629	20.874
X16	4.336	2.854	0.245	0.702	4.116	26.808
X15	1.161	1.232	3.272	-0.097	2.703	23.289
X17	2.575	4.210	0.048	-1.737	2.649	20.824
X6	-0.912	1.116	3.538	0.351	1.909	23.800

Table 21: Random Forest (5 least important features removed) Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	130	1	2	19
B	1	134	0	9
C	1	0	142	11
D	8	3	3	89

The random forest performed well without any configuration. feature selection was not effective. still struggled at seperating class D. mtry was tuned.

Table 22: Random Forest (5 least important features removed) Overall Statistics

Statistic	Value
Accuracy	0.8951
Kappa	0.8598
AccuracyLower	0.8665
AccuracyUpper	0.9194
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 23: Random Forest (5 least important features removed) Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9286	0.9710	0.9660	0.6953
Specificity	0.9467	0.9759	0.9704	0.9671
Pos Pred Value	0.8553	0.9306	0.9221	0.8641
Neg Pred Value	0.9751	0.9902	0.9875	0.9133
Precision	0.8553	0.9306	0.9221	0.8641
Recall	0.9286	0.9710	0.9660	0.6953
F1	0.8904	0.9504	0.9435	0.7706
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2351	0.2423	0.2568	0.1609
Detection Prevalence	0.2749	0.2604	0.2785	0.1863
Balanced Accuracy	0.9377	0.9735	0.9682	0.8312

Table 24: Random Forest (5 least important features removed) Feature Importance

	A	B	C	D	MeanDecreaseAccuracy	MeanDecreaseGini
X7	61.055	177.557	55.712	14.925	113.337	397.720
X8	88.792	25.710	73.925	-7.703	87.006	278.636
X10	55.157	28.584	65.397	21.244	78.331	153.548
X11	49.151	11.713	46.621	30.848	64.485	124.809
X9	37.606	31.477	56.004	17.270	64.405	251.544
X3	26.990	27.271	31.846	13.963	45.209	109.174
X2	21.793	13.329	13.399	9.146	25.922	60.077
X1	8.169	13.426	13.781	15.236	23.701	52.354
X4	17.311	5.883	-0.227	9.381	18.843	41.735
X5	13.363	2.294	-0.812	12.958	16.715	39.172
X14	10.922	-0.500	6.611	6.240	12.783	37.323
X13	7.942	0.018	8.406	4.494	10.923	30.103
X12	4.915	-0.774	6.641	6.986	9.581	30.379
X18	7.242	-1.901	4.388	2.293	6.436	29.636
X16	4.754	2.667	0.493	2.047	4.961	28.217

Table 25: Random Forest (5 least important features removed) Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	128	2	1	19
B	2	134	0	8
C	2	0	143	12
D	8	2	3	89

Table 26: Random Forest (5 least important features removed) Overall Statistics

Statistic	Value
Accuracy	0.8933
Kappa	0.8574
AccuracyLower	0.8645
AccuracyUpper	0.9178
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 27: Random Forest (5 least important features removed) Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9143	0.9710	0.9728	0.6953
Specificity	0.9467	0.9759	0.9655	0.9694
Pos Pred Value	0.8533	0.9306	0.9108	0.8725
Neg Pred Value	0.9702	0.9902	0.9899	0.9135
Precision	0.8533	0.9306	0.9108	0.8725
Recall	0.9143	0.9710	0.9728	0.6953
F1	0.8828	0.9504	0.9408	0.7739
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2315	0.2423	0.2586	0.1609
Detection Prevalence	0.2712	0.2604	0.2839	0.1844
Balanced Accuracy	0.9305	0.9735	0.9692	0.8324

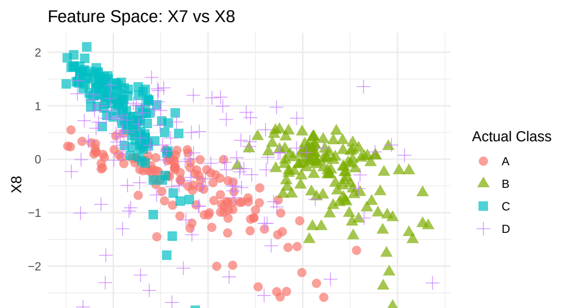
Table 28: Random Forest (5 least important features removed) Feature Importance

	A	B	C	D	MeanDecreaseAccuracy	MeanDecreaseGini
X7	49.378	124.698	53.309	12.102	97.825	384.170
X9	47.157	35.396	111.489	16.624	87.840	334.826
X10	52.393	24.362	55.209	21.704	66.744	146.548
X11	42.960	13.588	43.828	31.389	59.129	123.378
X3	26.132	36.587	35.320	14.353	50.914	123.061
X2	21.464	14.659	15.439	10.549	29.079	71.440
X1	9.646	14.493	15.000	15.215	26.767	66.584
X4	13.486	4.010	4.321	10.485	17.401	49.500
X5	12.570	3.156	-0.769	14.931	16.060	45.017
X14	11.881	2.210	7.208	9.348	14.554	43.894
X18	11.369	5.195	5.493	2.827	12.462	34.427
X13	6.973	-0.130	6.648	4.022	8.522	35.437
X12	2.292	0.042	8.676	4.968	8.161	34.572
X16	3.370	4.143	2.393	5.357	7.315	33.664
X20	5.940	1.541	4.773	0.424	6.834	27.432
X19	4.392	3.560	4.815	0.037	6.577	26.463
X17	2.796	4.223	3.016	1.059	5.315	25.539
X6	1.709	0.087	4.610	0.832	3.575	30.082
X15	0.840	1.282	4.396	0.137	3.127	28.455

Table 29: Tuned Random Forest Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	129	1	2	18
B	2	134	0	9
C	1	0	141	8
D	8	3	4	93

3.1.3 SVM



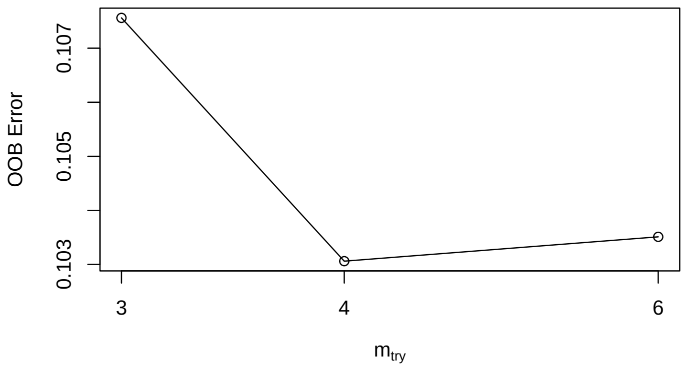


Figure 7: **Optimal mtry for Random Forest**

Table 30: Tuned Random Forest Overall Statistics

Statistic	Value
Accuracy	0.8987
Kappa	0.8647
AccuracyLower	0.8705
AccuracyUpper	0.9226
AccuracyNull	0.2658
AccuracyPValue	0.0000

Table 31: Tuned Random Forest Statistics by Class

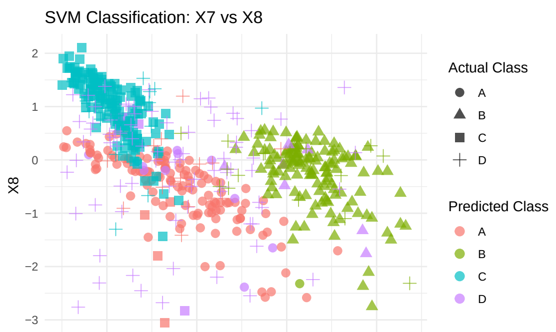
Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9214	0.9710	0.9592	0.7266
Specificity	0.9492	0.9735	0.9778	0.9647
Pos Pred Value	0.8600	0.9241	0.9400	0.8611
Neg Pred Value	0.9727	0.9902	0.9851	0.9213
Precision	0.8600	0.9241	0.9400	0.8611
Recall	0.9214	0.9710	0.9592	0.7266
F1	0.8897	0.9470	0.9495	0.7881
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2333	0.2423	0.2550	0.1682
Detection Prevalence	0.2712	0.2622	0.2712	0.1953
Balanced Accuracy	0.9353	0.9723	0.9685	0.8456

Table 32: SVM Performance with Different Kernels

Kernel	Accuracy	Kappa	F1 Score (A)	F1 Score (B)	F1 Score (C)	F1 Score (D)
Linear	0.8662	0.8212	0.8990	0.9155	0.9211	0.6926
Polynomial - Order 3	0.8463	0.7944	0.8562	0.9143	0.9145	0.6481
Polynomial - Order 5	0.7324	0.6415	0.6974	0.8276	0.8159	0.5393
Polynomial - Order 7	0.5371	0.3797	0.5404	0.5810	0.6271	0.3268
Radial	0.8843	0.8452	0.9048	0.9371	0.9346	0.7182
Sigmoid	0.7125	0.6159	0.7260	0.7823	0.8383	0.4583

Table 33: SVM Tuned Model Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	133	0	2	19
B	0	134	0	14
C	0	0	143	16
D	7	4	2	79



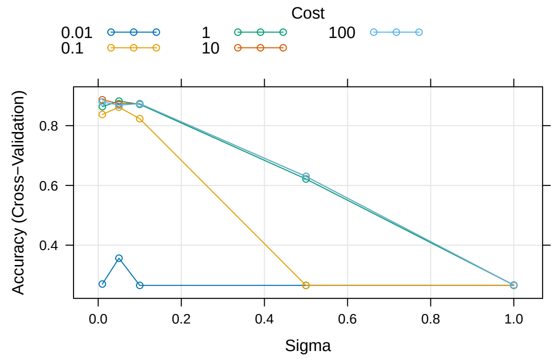


Figure 8: **Hyperparameter Affect on SVM Performance**

Table 34: SVM Tuned Model Overall Statistics

	Statistic	Value
Accuracy	Accuracy	0.9042
Kappa	Kappa	0.8719
AccuracyLower	AccuracyLower	0.8765
AccuracyUpper	AccuracyUpper	0.9274
AccuracyNull	AccuracyNull	0.2658
AccuracyPValue	AccuracyPValue	0.0000
McnemarPValue	McnemarPValue	NaN

Table 35: SVM Tuned Model Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9071	0.9493	0.9592	0.6172
Specificity	0.9564	0.9590	0.9507	0.9529
Pos Pred Value	0.8759	0.8851	0.8758	0.7980
Neg Pred Value	0.9681	0.9827	0.9847	0.8921
Precision	0.8759	0.8851	0.8758	0.7980
Recall	0.9071	0.9493	0.9592	0.6172
F1	0.8912	0.9161	0.9156	0.6960
Prevalence	0.2532	0.2495	0.2658	0.2315
Detection Rate	0.2297	0.2369	0.2550	0.1429
Detection Prevalence	0.2622	0.2676	0.2911	0.1790
Balanced Accuracy	0.9318	0.9542	0.9550	0.7851

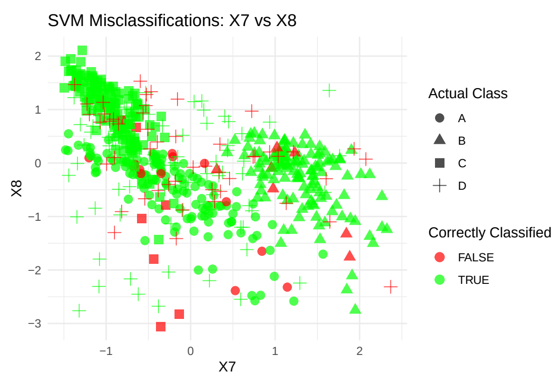


Figure 11: **Misclassifications - X7 by X8**

svm was good but not as good as random forest. lots of tuning. feature selection was uneffective

3.2 Unsupervised Learning Results

3.2.1 Agglomerative Hierarchical Clustering

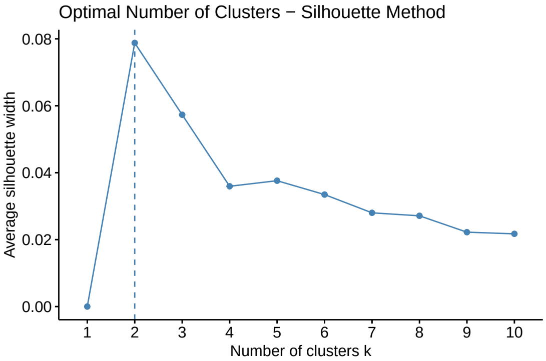


Figure 12: **Silhouette Plot**

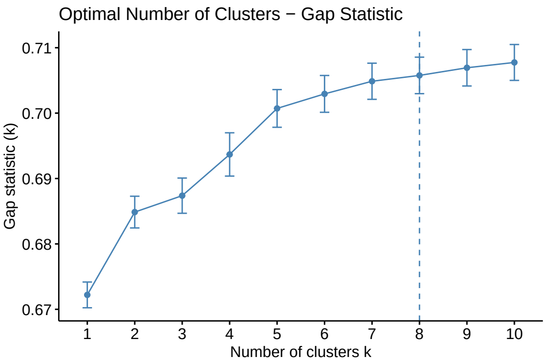


Figure 13: **Gap Statistic Plot**

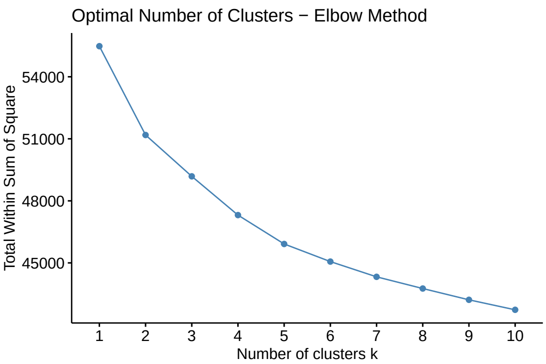


Figure 14: **Elbow Graph**

ahc was good not great.

3.2.2 Gaussian Mixed Model Clustering

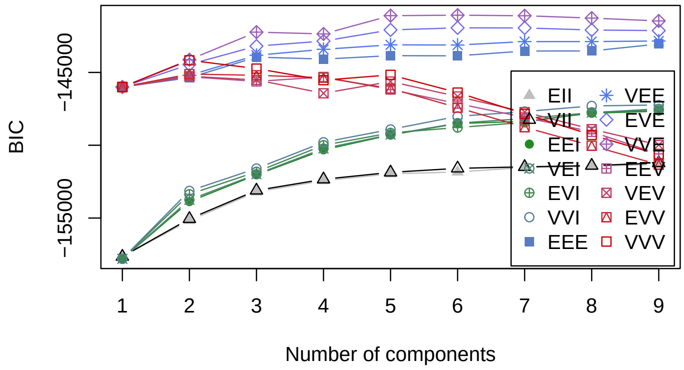
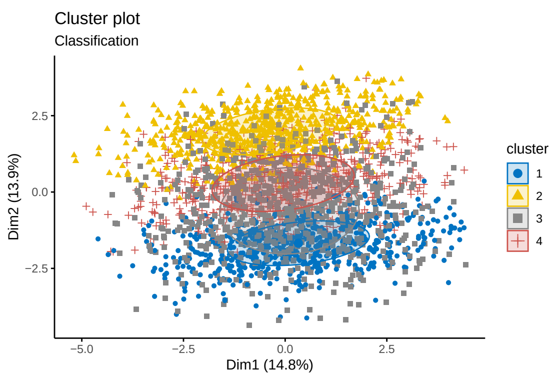


Figure 15: **Baysian Information Criteria Plot for Choosing Optimal Model**

VVE was found to be the best performing model. Promisingly, the plot peaked at four clusters. Since it is known that there actually are four categorical classes in the original data, this shows that the model is learning from the distributions of all four labels. Since we saw such overlap in classes and many misclassifications in class D previously, it would have been unsuprising to see three as the optimum number of classes,suggesting that data was better described by three categories than four.

In heirarchical clustering, we didn't see clear confirmation of four groups from the graphs using either the elbow method, gap method, or silloette method.

The three letters in the model name describe the shape, orientation, and orientation of the clusters that the model predicts("Initialisation" n.d.). In this case, VVE indicates that the model is predicting clusters that are elipsoids of equal orientation, but varying volume("mclustModelNames: MCLUST Model Names in Mclust: Gaussian Mixture Modelling for Model-Based Clustering, Classification, and Density Estimation" n.d.). This makes sense based on the PCA scatterplots where the data is shown as three roughly equally size elipses one above the other, with a fourth, larger elipses overlaying them, with the major axis of all four being roughly horizontal (along the first principle component)



This looks really similar to the original PCA plot (Figure 4), with class A B and C seperated and class D overlapping all three.

Table 36: Gaussian Mixture Model Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	641	0	0	62
B	0	662	0	38
C	0	0	677	18
D	61	32	60	524

Table 37: Gaussian Mixture Model Overall Statistics

	Statistic	Value
Accuracy	Accuracy	0.9023
Kappa	Kappa	0.8698
AccuracyLower	AccuracyLower	0.8907
AccuracyUpper	AccuracyUpper	0.9131
AccuracyNull	AccuracyNull	0.2656
AccuracyPValue	AccuracyPValue	0.0000

Table 38: Gaussian Mixture Model Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9131	0.9539	0.9186	0.8162
Specificity	0.9701	0.9817	0.9912	0.9283
Pos Pred Value	0.9118	0.9457	0.9741	0.7740
Neg Pred Value	0.9706	0.9846	0.9712	0.9438
Precision	0.9118	0.9457	0.9741	0.7740
Recall	0.9131	0.9539	0.9186	0.8162
F1	0.9125	0.9498	0.9455	0.7945
Prevalence	0.2530	0.2501	0.2656	0.2314
Detection Rate	0.2310	0.2386	0.2440	0.1888
Detection Prevalence	0.2533	0.2523	0.2505	0.2440
Balanced Accuracy	0.9416	0.9678	0.9549	0.8722

Gaussian mixed model clustering performed very well. It immediately had overall accuracy comparible with random forest, and performed notebly better than other models at seperating the fourth class successfully, with an F1 score of 0.8.

Attempts were made to further tune the model through regularization:

Table 39: Gaussian Mixture Model Performance with Different Regularization Settings

Model	ARI	Accuracy	F1 Score (A)	F1 Score (B)	F1 Score (C)	F1 Score (D)
No regularization	0.7702	0.9023	0.9125	0.9498	0.9455	0.7945
Shrinkage = 0.001	0.6016	0.7643	0.8096	0.8876	0.8795	0.1837
Shrinkage = 0.01	0.5611	0.7571	0.7984	0.8995	0.8768	NA
Shrinkage = 0.05	0.5770	0.7575	0.7995	0.8977	0.8789	NA
Shrinkage = 0.1	0.6016	0.7643	0.8096	0.8876	0.8795	0.1837
Shrinkage = 0.5	0.5640	0.7575	0.7984	0.8995	0.8783	NA

The best performing model did not use regularization.

The motivation for using regularization is to help the model perform better when using features that correlate together. Since regularization in fact hindered performance, another approach is to use factor analysis to reduce the dimensionality of the data. Factor analysis is suitable because it is suited to handling the groups of correlated features notable in the EDA results, and because the data is biological in origin. With biological data, there is often an underlying cause, like a gene expression, that can have many measurable implecations, like disease symptoms or physical characteristics. Because we know these mechanisms may exist in the source of our data, factor analysis is a good choice to reduce dimensions while preserving as much information as possible.

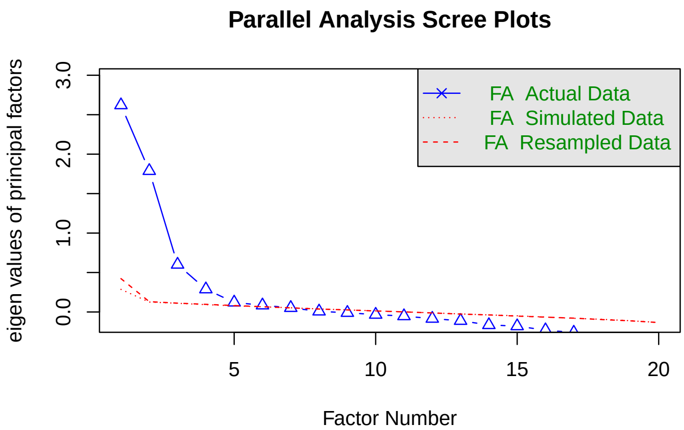


Figure 16: **Scree Plot:** *for Choosing Number of Factors for Dimensionality Reduction*

The parallel analysis results suggest that the optimum number of factors is 6. Using the maximum likelyhood method finds the number of factors where the value of the eigenvalue is above what would be expected by random chance.

The underlying values from the analysis show that the first two factors contribute the most, with a sharp drop after that. So a dimensionality reduction to two factors could be a reasonable option. We can also see that the seventh and eigth eigenvalues are not much smaller than the sixth, so swapping some of the smaller factors could also be a justifyable experiment.

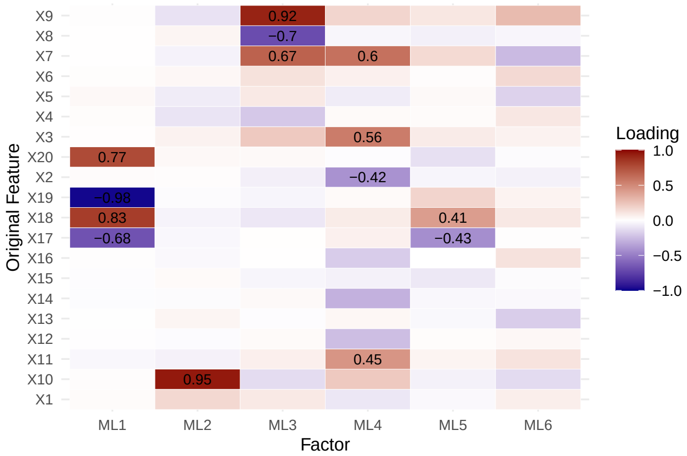


Figure 17: **Factor Analysis Loadings:** *loadings values by feature*

The loadings of features to factors shows the highly correlated features in the correlated groups are all heavily loaded to the same factor, which will succesfully decrease the multicolinearily present in the dataset.

Table 40: Gaussian Mixture Model Confusion Matrix

	Reference			
Prediction	A	B	C	D
A	650	4	1	77
B	10	669	0	37
C	2	0	728	41
D	40	21	8	487

Table 41: Gaussian Mixture Model Overall Statistics

	Statistic	Value
Accuracy	Accuracy	0.9023
Kappa	Kappa	0.8698
AccuracyLower	AccuracyLower	0.8907
AccuracyUpper	AccuracyUpper	0.9131
AccuracyNull	AccuracyNull	0.2656
AccuracyPValue	AccuracyPValue	0.0000

Operating on the factors rather than the original features, overall accuracy is slightly higher and the balanced accuracy of cluster 3 (class D) is slightly lower. This is a trade off that requires more knowledge of the intended use of the model to make a choice between the two.

However, the fact that factor analysis leads to increased accuracy overall while decreasing the dimensionality of the data so far is an interesting finding.

Table 42: Gaussian Mixture Model Statistics by Class

Statistic	Class: A	Class: B	Class: C	Class: D
Sensitivity	0.9259	0.9640	0.9878	0.7586
Specificity	0.9604	0.9774	0.9789	0.9677
Pos Pred Value	0.8880	0.9344	0.9442	0.8759
Neg Pred Value	0.9745	0.9879	0.9955	0.9301
Precision	0.8880	0.9344	0.9442	0.8759
Recall	0.9259	0.9640	0.9878	0.7586
F1	0.9066	0.9489	0.9655	0.8130
Prevalence	0.2530	0.2501	0.2656	0.2314
Detection Rate	0.2342	0.2411	0.2623	0.1755
Detection Prevalence	0.2638	0.2580	0.2778	0.2004
Balanced Accuracy	0.9432	0.9707	0.9833	0.8631

3.2.2.1 Model Evaluation

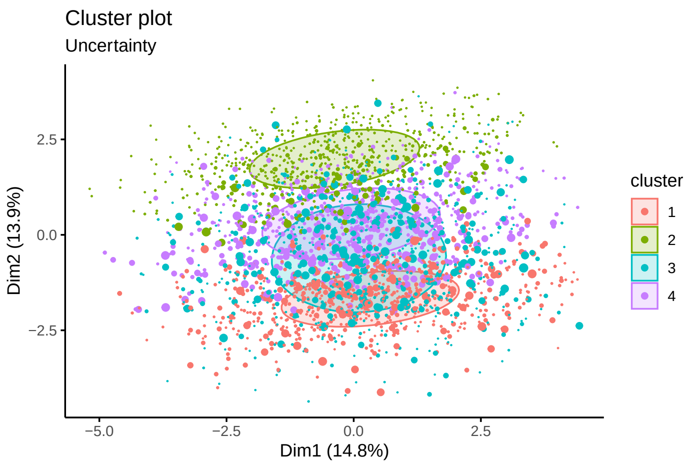


Figure 18: **Cluster Uncertainty Plot:** *Clusters shown with uncertain points by size*

Table 43: High Uncertainty points in each Class

cluster	true_label	n	percentage
1	B	662	94.5714
1	D	38	5.4286
2	C	677	97.4101
2	D	18	2.5899
3	D	524	77.4003
3	A	61	9.0103
3	C	60	8.8626
3	B	32	4.7267
4	A	641	91.1807
4	D	62	8.8193

3.3 Bootstrapping

Bootstrapping was used to oversample the original dataset and increase the number of observations to 10,000. With a test-train split of 70%-30%, the model were trained on 7000 observations for supervised methods. All three models recieve a modest bump in overall accuracy and in the Class D F1 score (*table 41*).

This shows that the performance of any model can be improved without any further tuning by collecting a larger dataset from the population. However, the increases are small and it is very likely that the cost of collecting more data is prohibative given the performance gains that this analysis suggests can be expected. Given this finding, it is recommended that further research focuses on improved modelling techniques, or the collection data with new descriptive features.

In unsupervised learning, the bootstrapping method leads to poor performance from the model based model, which now suggests an optimal number of clusters of 8. The duplication caused by boostrapping violates the assumptions that underpin the gaussian mixture model approach because it is assumed that the data is all drawn from the population produced by normal distribuitions. This means that bootstrapping is not appropriate for testing the performance of this model on a larger dataset.

Table 44: Comparison of Model Performance on Bootstrapped Data

	Original Data		Bootstrapped Data	
Model	Accuracy	Class D F1 Score	Accuracy	Class D F1 Score
Logistic Regression	0.8571	0.6725	0.8618	0.7193
Support Vector Machines	0.8626	0.6930	0.9103	0.8024
Random Forest	0.8987	0.7881	0.9141	0.8171

4 Discussion

The relative performance of different learning models can give some insight to the underlying structure of the data. If random forest and agglomerative hierarchical clustering performed significantly better than the other models, this would be evidence that the data was heirarchical in nature. If logistic regression performed as well as any of the other models, it would indicate that the data was extremely structured and easy to model, with strong linear relationships between the predictive features and the labels. The results found that random forest performed best followed by the radial kernel SVM. Both of these algorithms are better at modelling with non-linear relationships. This suggests that the the relationship between the features and the label exhibits some non-linearity.

There are also lessons learned from the challenge of seperating class D which arrose in every model that was implemented. Class D was harder for every model to correctly predict. In the context of biological data there are a several explainations of why this would happen, including that D is a super-class in a hierarchy where the other three are sub classes. This appears unlikely due to the underperformance of agglomerative hierarchical clustering. Another explaination is that D could be a transition state between other classes. This is not supported by the visualization of the data where class D seems to form a differently shaped sigmoid to the other classes, and overlap all three. Its still possible that D represents an immature state that will later develop into one of the other three. The uncertainy of the predictions of this class by an array of models with such different underlying principles suggests that the difficulty is not a limitation of any algorithm, and it is likely that the features of the data do not have the predictive power necessary for efficient seperation of class D. Better performing models could be developed if data was collected with additional variables.

At the outset of the project, it was reasonable to assume that the supervised learning would out-perform the unsupervised learning models. The gaussian mixture model achieving the highest performance metrics speaks to the high amounts of noise present within each cluster and the dataset as a whole, as performing well on these kinds of data is a hallmark of the gmm algorithm.

Factor analysis had some success as the gmm model was able to retain its high accuracy and increase the F1 score of class D with a considerable reduction in the dimensionality of the data, suggesting the existance of underlying biological mechanisms or environmental factors that lead to the observed features arising. Although well performing statistical models were trained using the features and the factor analysis approach, better results mights be possible using the underlying factors themselves if it is possible to measure them.

The best models had good overall accuracy but caution is advised for implementing any model with this data due to the underperformance in class D - if false positives or false negatives in this class have serious implications, some models become immediately unusable. Examples were this would be the case include when the classes represent risk of side effects to a medication option.

5 Conclusion

The results of this project demonstrate how advanced statistical techniques are relevent to fields of research using biological data, with the potential for powerful models evident. The gaussian mixed model would be the one model that could be taken forward for use classifying new data collected or for generalizing to another data set because of its high overall accuracy but particularly because of its high balanced accuracy in class D compared to other models. The greatest limitation of the models trained in this project is the underperformance of classifying class D, which could be critical in some applications. The collection of data with more descriptive features could allow for the development of more powerful models using the same techniques, or the work in this project could be build upon to create more refined models that perform better for specific use cases. Further research should focus on improving the results of the random forest and gaussian mixture model approaches. An algorithm such as A gradient boosting algorithm such as XGBoost or lightGBM would build upon the successful tree-based approach of random forest. With each tree in a gradient boosting approach able to learn from the one before and the high tunability of the model, a model that performs better at the shortcomings of models in this report is possible (Chen and Guestrin 2016). An exciting direction to take forward the success of gmm would be to experiment with semi-supervised learning. A sample of class labels supplied to a model based clustering algorithm to guide the initial assignment of cluster can lead to learning distributions that are more specific to each cluster, with better results (Géron 2023).

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