

1 **Age Grading *An. Gambiae* and *An. Arabiensis* Using Near** 2 **Infrared Spectra and Artificial Neural Networks**

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20 **Abstract**

21 **Background**

22 Near infrared spectroscopy (NIRS) is currently complementing techniques to age-grade
23 mosquitoes. NIRS classifies lab-reared and semi-field raised mosquitoes into $<$ or ≥ 7 days old with
24 an average accuracy of 80%, achieved by training a regression model using partial least squares
25 (PLS) and interpreted as a binary classifier.

26 **Methods and findings**

27 We explore whether using an artificial neural network (ANN) analysis instead of PLS
28 regression improves the current accuracy of NIRS models for age-grading malaria transmitting
29 mosquitoes. We also explore if directly training a binary classifier instead of training a regression
30 model and interpreting it as a binary classifier improves the accuracy.

31 A total of 786 and 870 NIR spectra collected from laboratory reared *An. gambiae* and *An.*
32 *arabiensis*, respectively, were used and pre-processed according to previously published protocols.
33 Based on ten-fold Monte Carlo cross-validation, an ANN regression model scored root mean
34 squared error (RMSE) of 1.6 ± 0.2 for *An. gambiae* and 2.8 ± 0.2 for *An. arabiensis*; whereas the
35 PLS regression model scored RMSE of 3.7 ± 0.2 for *An. gambiae*, and 4.5 ± 0.1 for *An.*
36 *arabiensis*. When we interpreted regression models as binary classifiers, the accuracy of the ANN
37 regression model was $93.7 \pm 1.0 \%$ for *An. gambiae*, and $90.2 \pm 1.7 \%$ for *An. arabiensis*; while
38 PLS regression model scored the accuracy of $83.9 \pm 2.3\%$ for *An. gambiae*, and $80.3 \pm 2.1\%$ for
39 *An. arabiensis*. We also find that a directly trained binary classifier yields higher age estimation
40 accuracy than a regression model interpreted as a binary classifier. A directly trained ANN binary
41 classifier scored an accuracy of 99.4 ± 1.0 for *An. gambiae*, and $99.0 \pm 0.6\%$ for *An. arabiensis*;
42 while a directly trained PLS binary classifier scored $93.6 \pm 1.2\%$ for *An. gambiae*, and $88.7 \pm$
43 1.1% for *An. arabiensis*.

44 **Conclusion**

45 Training both regression and binary classification age models using ANNs yields models with
46 higher estimation accuracies than when the same age models are trained using PLS. Regardless of
47 the model architecture, directly trained binary classifiers score higher accuracy on classifying age of
48 mosquitoes than a regression model translated as binary classifier. Therefore, we recommend
49 training models to estimate age of *An. gambiae* and *An. arabiensis* using ANN model architectures
50 and direct training of binary classifier instead of training a regression model and interpret it as a
51 binary classifier.

52 Introduction

53 Estimating the age of mosquitoes is one of the indicators used by entomologists for estimating
54 vectorial capacity [1] and the effectiveness of an existing mosquito control intervention. Malaria is a
55 vector-borne parasitic disease transmitted to people by mosquitoes of the genus *Anopheles*. The
56 disease killed approximately 445,000 people in 2016 [2]. Mosquitoes contribute to malaria
57 transmission by hosting and allowing the development to maturity of the malaria-causing
58 *Plasmodium* parasite [3]. Depending on environmental temperature, *Plasmodium* takes 10-14 days
59 in an *Anopheles* mosquito to develop fully enough to be transmitted to humans [3]. Therefore,
60 knowing the age of a mosquito provides an indication of whether a mosquito is capable of
61 transmitting malaria.

62 Knowing the age of a mosquito population is also important when evaluating the effectiveness
63 of a mosquito control intervention. Commonly used vector control interventions such as insecticide
64 treated nets (ITNs) and indoor residual spraying (IRS) reduce the abundance and the lifespan of a
65 mosquito population to a level that does not support *Plasmodium* parasite development to maturity
66 [4, 5]. Monitoring and evaluation of ITNs and IRS involves determining the age and species
67 composition of the mosquito population before and after intervention. The presence of a small
68 number of old mosquitoes in an area with an (ITNs or IRS) intervention indicates that the
69 intervention is working. On the other hand, if there are more old mosquitoes, the intervention is not
70 working effectively.

71 The current techniques used to estimate mosquito age are based on a combination of ovary
72 dissecting and conventional microscopy to determine their egg laying history. Those found to have
73 laid eggs are assumed to be older than those found to not have laid eggs [6]. This assumption can be
74 misleading, as mosquitoes can be old but have not laid eggs and can be young (at least three days
75 old), and have laid eggs. Dissection is laborious, difficult, and limited to only few experts. As a
76 result, we need a new approach to address these limitations.

77 Different techniques such as a change in abundance of cuticular hydrocarbons [7, 8],
78 transcriptional profiles [9, 10], and proteomics [11, 12] have been developed to age grade *Anopheles*
79 mosquitoes. However, these techniques are still in early development stages and are limited to
80 analyzing a small number of samples due to high analysis costs involved.

81 Near infrared spectroscopy (NIRS) is a complementary method to the current mosquito age
82 grading techniques [13, 14]. NIRS is a high throughput technique, which measures the amount of the
83 near infrared energy absorbed by samples. NIRS has been applied to identify species of insects
84 infecting stored grains [15]; to age grade houseflies [16], stored-grain pests [17], and biting midges
85 [18]; to differentiate between species and subspecies of termites [19]; to estimate the age and to

86 identify species of morphologically indistinguishable laboratory reared and semi-field raised
87 *Anopheles gambiae* and *Anopheles arabiensis* mosquitoes [13, 14, 20-23]; to estimate the age of
88 *Aedes aegypti* mosquitoes [24]; and to detect and identify two strains of *Wolbachia pipientis*
89 (wMelPop and wMel) in male and female laboratory-reared *Aedes aegypti* mosquitoes [25].

90 The current state-of-the-art of the accuracy of NIRS to classify the age of lab-reared *An.*
91 *gambiae* and *An. arabiensis* is an average of 80% [13, 14, 20-23]. This accuracy is based on a
92 trained regression model using partial least squares (PLS) and interpreted as a binary classifier to
93 classify mosquitoes into two age groups (< 7 days and ≥ 7 days).

94 In this paper, using a set of spectra collected from lab-reared *An. gambiae* and *An. arabiensis*,
95 we explored ways to improve the reported accuracy of a PLS model for estimating age of malaria-
96 transmitting mosquitoes. Selection of a method to train a model is one of the important factors
97 influencing the accuracy of the model [26]. Studies [27-30] compared the accuracies of artificial
98 neural network (ANN) and PLS regression models for predicting respiratory ventilation; explored
99 the application of ANN and PLS to predict the changes of anthocyanins, ascorbic acid, total phenols,
100 flavonoids, and antioxidant activity during storage of red bayberry juice; determined glucose
101 multivariation in whole blood using partial least-squares and artificial neural networks based on
102 mid-infrared spectroscopy; and compared modeling of nonlinear systems with artificial neural
103 networks and partial least squares, concluding that ANN models generally perform better than PLS
104 models. Therefore, using ANN [29-31] and PLS, we trained regression age models and compared
105 results.

106 Since previous studies [13, 14, 20-23] trained a regression model and interpreted it as a binary
107 classifier (< 7 d and ≥ 7 d), the interpretation process may introduce errors and compromise the
108 accuracy of the model. We further trained ANN and PLS binary classifiers and compared their
109 accuracies with the ANN and PLS regression models translated as binary classifiers.

110 We find that training of both regression and binary classification models using an artificial
111 neural network architectures yields higher accuracies than when the corresponding models are
112 trained using partial least squares model architectures. Also, regardless of the architecture of the
113 model, training a binary classifier yields higher age class estimation accuracy than a regression
114 model interpreted as a binary classifier.

115

116 **Materials and methods**

117 **Ethics approval**

118 Permission for blood feeding laboratory-reared mosquitoes was obtained from the Ifakara
119 Health Institute (IHI) Review Board, under Ethical clearance No. IHRDC/EC4/CL.N96/2004. Oral
120 consent was obtained from each adult volunteer involved in the study. The volunteers were given the
121 right to refuse to participate or to withdraw from the experiment at any time.

122 **Mosquito and spectra collection**

123 We used spectra of *Anopheles gambiae* mosquito collected at 1, 3, 5, 7, 9, 11, 15, and 20
124 days and *An. arabiensis* collected at 1, 3, 5, 7, 9, 11, 15, 20 and 25 days post emergence from the
125 Ifakara Health Institute insectary. While *An. arabiensis* were reared in a semi-field system (SFS) at
126 ambient conditions, *An. gambiae* were reared in a room made of bricks at controlled conditions.
127 Adult mosquitoes were often provided with a human blood meal in a week and 10% glucose
128 solution daily. Using a LabSpec 5000 NIR spectrometer with an integrated light source (ASD Inc.,
129 Longmont, CO), we followed the protocol supplied by Mayagaya and colleagues to collect spectra
130 [13]. Prior to spectra collection, as opposed to killing by chloroform, mosquitoes were killed by
131 freezing for 20 minutes. A total of 786 *An. gambiae* and 870 *An. arabiensis* were scanned with at
132 least 70 mosquitoes from each age group.

133 **Model training**

134 We first trained ANN and PLS regression models, scored and compared their accuracies as
135 regressors and then as binary classifiers. We further trained binary classifiers and compared the
136 accuracies with regressors interpreted as binary classifiers. We used a two-tail t-test to test the
137 hypothesis that there is significant difference in accuracies between ANN and PLS trained model, a
138 one-tail t-test to test the hypothesis that an ANN trained model scores higher accuracies than a PLS
139 trained model.

140 In each species, we separately processed spectra according to Mayagaya et. al, randomized,
141 and divided processed spectra into two groups. The first group contained 70% of the total spectra
142 and was used for training models. The second group had 30% of the total spectra and was used for
143 out-of-sample testing.

144 We trained a PLS ten-components model on using ten-fold cross validation [32]. Even though
145 a range of six to ten PLS components were used in previous studies [13, 14, 20-22], we used ten
146 PLS components after plotting the percentage of variance explained in the dependent variable
147 against the number of PLS components (S1 Fig in the supporting information). For both species,

148 there is not much change in the percentage variance explained in the dependent variables beyond ten
149 components.

150 For the ANN model, we trained a feed-forward ANN with one hidden layer, ten neurons,
151 and a linear transfer function (purelin) using Levenberg-Marquardt (damped least-squares)
152 optimization [33]. We used actual mosquito ages as labels during training of both PLS and ANN
153 regression models. We determined whether the trained models are over-fit by applying trained
154 models (PLS and ANN) to estimate ages of mosquitoes on both training (in sample) and test (out-of-
155 sample) data sets. Normally, if the model is not over-fit, the accuracy of the model is consistent
156 between training and test sets [34].

157 The accuracies of the models were determined by computing their root mean squared error
158 (RMSE) [35-37]. We evaluated the influence of the model architecture on the model accuracy by
159 comparing their accuracies.

160 When interpreting the regression models as binary classifiers, mosquitoes with an estimated
161 age < 7 days were considered as less than seven days old, and those ≥ 7 were considered older than
162 or equal to seven days old. Using Equations 1, 2, and 3, we computed and compared sensitivity,
163 specificity, and accuracy between the PLS and ANN regression models interpreted as binary
164 classifiers. Sensitivity of the model is the ability to classify mosquitoes correctly, which are older
165 than or equal to seven days old (assumed to be positively related to malaria transmission), and
166 specificity is the ability of the model to classify mosquitoes correctly which are less than seven days
167 old (assumed to be negatively related to malaria transmission) [38-40].

168

169 Sensitivity =
$$\frac{\text{Number of mosquitoes correctly predicted as } \geq 7 \text{ days old}}{\text{Total number of mosquitoes } \geq 7 \text{ days old}} \quad (1)$$

170

171 Specificity =
$$\frac{\text{Number of mosquitoes correctly predicted } < 7 \text{ days old}}{\text{Total number mosquitoes } < 7 \text{ days old}} \quad (2)$$

172

173 Accuracy =
$$\frac{\text{Sensitivity} + \text{Specificity}}{\text{Total number of all mosquitoes in a test set}} \quad (3)$$

174

175 Training a regression model and interpreting it as a binary classifier can compromise the
176 accuracy of the model as a classifier. This is because, while training a regression model forces the
177 model to learn differences between actual ages of mosquitoes, direct training of a binary classifier
178 forces the model to learn similarities between mosquitoes of the same class and only differences
179 between two classes. Therefore, we directly trained binary classification models using ANN and

180 PLS architectures and compare the accuracies with the ANN and PLS regression models interpreted
181 as binary classifiers. In both species, we divided processed spectra (786 spectra for *An. gambiae* and
182 870 spectra for *An. arabiensis*) into two groups; < 7 days old and ≥ 7 days old. The spectra in a
183 group with mosquitoes < 7 days old were labeled 0, 1 for those in a group with mosquitoes ≥ 7 days
184 old, and the two groups were merged. The spectra were randomized and divided into training ($N =$
185 508 for both species) and test ($N = 278$ for *An. gambiae* and $N = 362$ for *An. arabiensis*) sets. We
186 trained a PLS ten-component model using ten-fold cross-validation [32] and a one hidden layer, ten
187 neuron feed-forward ANN using logistic regression as a transfer function and Levenberg-Marquardt
188 (damped least-squares) optimization for training [33, 41]. During interpretation of these models,
189 mosquitoes < 0.5 were considered as < 7 days old and ≥ 0.5 as ≥ 7 days old. Using Equations 1, 2,
190 and 3, for each species, we computed specificity, sensitivity, and accuracy of the trained PLS and
191 ANN binary classifiers and compared to the PLS and ANN regressors interpreted as the binary
192 classifiers.

193 We repeated the process of random splitting the dataset into training and test sets; training,
194 testing and scoring the accuracies of trained models ten times and compare the average results, a
195 process known as Monte Carlo cross-validation [42-44].

196 **Results**

197 Both PLS and ANN regression models consistently estimated the age of *An. gambiae* and *An.*
198 *arabiensis* in the training and test data sets, showing that the models were not over-fit during
199 training (Fig 1). Table 1, S2 Fig in the supporting information and Fig 2 present the performances of
200 PLS and ANN regression models when estimating actual age of *An. gambiae* and *An. arabiensis* in
201 the test data set and when their outputs are interpreted into two age classes, showing significant
202 differences in accuracies of the two models (PLS vs ANN models). Table 1 further shows that the
203 ANN regression model scores significantly higher accuracy than the PLS regression model.

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205

206 **Fig 1: PLS (A and C) and ANN (B and D) regression models, estimating actual age of training**
207 **and testing samples of *An. gambiae* (A and B) and *An. arabiensis* (C and D), respectively.**

208 **Table 1: Performance analysis of PLS and ANN regression models on estimating age of *An.***
 209 ***gambiae* and *An. arabiensis*. Results from ten-fold Monte Carlo cross-validation.**

Species	Model estimation	Metric	Model architecture		P-value (two tail)	P-value (one tail)
			PLS	ANN		
<i>An. gambiae</i>	Actual age	RMSE	3.7 ± 0.2	1.6 ± 0.2	3.9 x 10 ⁻⁰⁹	1.6 x 10 ⁻¹¹
	Age class	Accuracy (%)	83.9 ± 2.3	93.7 ± 1.0	3.6 x 10 ⁻⁰⁷	2.3 x 10 ⁻⁰⁷
		Sensitivity (%)	89.0 ± 2.1	92.5 ± 1.6	4.7 x 10 ⁻⁰²	4.7 x 10 ⁻⁰¹
		Specificity (%)	75.8 ± 5.2	95.6 ± 1.8	3.7 x 10 ⁻¹¹	1.1 x 10 ⁻⁰⁶
<i>An. arabiensis</i>	Actual age	RMSE	4.5 ± 0.1	2.8 ± 0.2	1.7 x 10 ⁻⁰⁹	5.9 x 10 ⁻⁰⁸
	Age class	Accuracy (%)	80.3 ± 2.1	90.2 ± 1.7	1.4 x 10 ⁻⁰⁷	2.4 x 10 ⁻⁰⁸
		Sensitivity (%)	90.5 ± 1.9	91.7 ± 3.3	5.8 x 10 ⁻⁰¹	6.0 x 10 ⁻⁰¹
		Specificity (%)	60.3 ± 4.2	88.4 ± 3.9	1.7 x 10 ⁻⁰⁷	1.2 x 10 ⁻⁰⁶

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213 **Fig 2: Number of *An. gambiae* s.s (A and B) and *An. arabiensis* (C and D) in two age classes**
 214 **(less than or greater/equal seven days) when PLS (A and C) and ANN (B and D) regression**
 215 **models, respectively, interpreted as binary classifiers.**

216

217

218 Fig 3 represents consistency in accuracy of PLS (A and C) and ANN (B and D) directly trained
 219 binary classifiers on estimating both training and test data sets, showing that the models were not
 220 over-fitted during training. Fig 4, S3 Fig in the supporting information and Table 2 present the
 221 results when directly trained PLS (A and C) and ANN (B and D) binary classifiers were applied to
 222 classify ages of *An. gambiae* (A and B) and *An. arabiensis* (C and D) in test sets (out-of-the sample
 223 testing), showing ANN binary classifier scores higher accuracy than the PLS binary classifier. The
 224 results further show that in both species, irrespective of the architecture used to train the model,

225 direct training of the binary classifier scores significantly higher accuracy, specificity, and
 226 sensitivity than the regression model translated as a binary classifier (S1 Table in the supporting
 227 information).

228

229

230 **Fig 3: The consistency in accuracies of directly trained PLS (A and C) and ANN (B and D)**
 231 **binary classifiers for estimating age classes of *An.gambiae* (A and B) and *An. arabiensis* (C and**
 232 **D) in both training and testing sets.**

233

234

235 **Fig 4: The number of correct and false predictions in each estimated age-class when directly**
 236 **trained PLS (A and C) and ANN (B and D) binary classifiers were applied to classify age of**
 237 ***An. gambiae* (A and B) and *An. arabiensis* (C and D) in testing sets. Results from ten replicates.**

238

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Table 2: Comparison of the accuracy of ANN and PLS classification models on ten replicates

Species	Metric	Model architecture		P-value (two-tail)	P-value (one-tail)
		PLS	ANN		
<i>An. gambiae</i>	Accuracy (%)	93.6 ± 1.2	99.4 ± 1.0	2.4 x 10 ⁻¹⁹	1.2 x 10 ⁻¹⁹
	Sensitivity (%)	94.4 ± 1.6	99.3 ± 1.4	1.6 x 10 ⁻⁰⁴	2.0 x 10 ⁻⁰⁵
	Specificity (%)	92.4 ± 1.9	99.5 ± 0.7	2.2 x 10 ⁻⁰⁶	6.0 x 10 ⁻⁰⁵
<i>An. arabiensis</i>	Accuracy (%)	88.7 ± 1.1	99.0 ± 0.6	1.5 x 10 ⁻²¹	7.6 x 10 ⁻²²
	Sensitivity (%)	95.4 ± 1.4	99.5 ± 0.5	4.5 x 10 ⁻⁰⁵	2.3 x 10 ⁻⁰⁵
	Specificity (%)	75.2 ± 3.4	98.3 ± 1.3	4.0 x 10 ⁻⁰⁹	2.0 x 10 ⁻⁰⁹

240

241

242 Discussion

243 This study aimed at improving the current state of the art accuracies of the models trained using near
 244 infrared spectra to estimate the age of *An. gambiae* and *An. arabiensis*. Previous studies [13, 14, 20-
 245 23] trained a regression model using partial least squares (PLS) and interpreted it as a binary
 246 classifier (< 7 d and ≥ 7 d) with an accuracy around 80%.

247 Knowing that the selection of a model architecture often influences the model accuracy [26],
248 we trained age regression models using an artificial neural network [29-31, 45, 46] and partial least
249 squares as model architectures and compared the accuracies. ANN models achieved significantly
250 higher accuracies than corresponding PLS regression models. As summarized in Table 1, ANN
251 regression models scored an average RMSE of 1.60 ± 0.18 for *An. gambiae* and 2.81 ± 0.22 for
252 *An. arabiensis*. The PLS regression models scored RMSE of 3.66 ± 0.23 for *An. gambiae* and 4.49
253 ± 0.09 for *An. arabiensis*. When both ANN and PLS regression models were interpreted as binary
254 classifiers, ANN regression model scored accuracy, sensitivity, and specificity of $93.71 \pm 1.03\%$,
255 $92.54 \pm 1.60\%$, and $95.64 \pm 1.82\%$, respectively, for *An. gambiae*; $90.16 \pm 1.70\%$, $91.68 \pm$
256 3.27% and $88.44 \pm 3.86\%$, respectively, for *An. arabiensis*. The PLS regression model scored
257 accuracy, sensitivity, and specificity of $83.85 \pm 2.32\%$, $89.00 \pm 2.10\%$, and $75.82 \pm 5.22\%$,
258 respectively, for *An. gambiae*; $80.30 \pm 2.06\%$, $90.48 \pm 1.88\%$, and $60.25 \pm 4.20\%$, respectively,
259 for *An. arabiensis*.

260 The interpretation of a regression model into a binary classifier can introduce errors that
261 compromise the accuracy of the model. We directly trained PLS and ANN binary classifiers and
262 compared the accuracies with ANN and PLS regression models interpreted as binary classifiers.
263 Irrespective of the model architecture, directly trained binary classifiers scored significantly higher
264 accuracies than corresponding regression models interpreted as binary classifiers (S1 Table in the
265 supporting information). The explanation of these results could be that, training a regression model
266 and interpreting it as a binary classifier involved learning differences between multiple age groups
267 (1, 3, 5, 7, 9, 11, 13, 15, and 20 days old for *An. gambiae* and 1, 3, 5, 7, 9, 11, 13, 15, 20 and 25 days
268 for *An. arabiensis*) of mosquitoes, which can be challenging for two consecutive age groups. In
269 contrast, direct training of the binary classifier involved learning differences existing between only
270 two age groups. During direct training of the binary classifier, the process of dividing spectra into
271 two groups (< 7 or ≥ 7 days) forced a model to learn similarities instead of differences between
272 mosquitoes of the same age class. We also observed that directly trained ANN binary classifier
273 scored higher accuracy than directly trained PLS binary classifier. ANN binary classifier scored an
274 accuracy, sensitivity, and specificity of $99.4 \pm 1.0\%$, $99.3 \pm 1.4\%$, and $99.5 \pm 0.7\%$,
275 respectively, for *An. gambiae*; $99.0 \pm 0.6\%$, $99.5 \pm 0.5\%$, and $98.3 \pm 1.3\%$, respectively, for *An.*
276 *arabiensis*. The PLS binary classifier scored $93.6 \pm 1.2\%$, $94.4 \pm 1.6\%$, and $92.5 \pm 1.9\%$ for *An.*
277 *gambiae*; $88.7 \pm 1.1\%$, $95.5 \pm 1.4\%$, and $75.2 \pm 3.5\%$ for *An. arabiensis* (Table 2).

278 Our study is not the first to observe ANN model outperforming PLS model. These findings
279 are supported with other previous studies [27-29, 31] compared the accuracies of ANN and PLS
280 models, where they report ANN perform better than PLS. The explanation on these results could be

281 that ANN, unlike PLS, considers both linear and unknown non-linear relationships between
282 dependent and independent variables [29-31]; builds independent-dependent relationships that
283 interpolates well even to cases that were not exactly presented by training data; and has a self
284 mechanism of filtering and handling noisy data during training [45, 46]. Hence, ANN models are
285 unbiased estimators in contrast to PLS models (Fig 5 and S4 Fig in supporting information).

286

287

288 **Fig 5. Error distribution per actual age of *An. gambiae* and *An. arabiensis* when ANN and PLS**
289 **regressors applied to estimate the actual ages of mosquitoes in training and test data sets,**
290 **showing uniform distribution of errors (un-biased estimating) across actual ages of mosquitoes**
291 **for ANN regressor and un-uniform distribution of errors (biased estimating) for PLS**
292 **regressor.**

293

294

295 **Conclusion**

296 We conclude that training both regression and binary classification age artificial neural network
297 models yield higher accuracies than partial least squares models. Also, training a binary classifier
298 scores higher accuracy than training a regression model and interpreting it as a binary classifier.
299 Hence, we recommend training of age models using artificial neural network and training of binary
300 classifier instead of training regression model and interpret it as binary classifier.

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310

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- 435

436 **Supporting information**

437 **S1 Fig. The percentage of variance explained in the dependent variable against the number of**
438 **PLS components: A) *An. gambiae* B) *An. arabiensis* (TIFF)**

439

440 **S2 Fig. Box plot showing the performance of ANN and PLS regression models when applied to**
441 **estimate the actual ages of *An. gambiae* and *An. arabiensis* in the test data set. (TIFF)**

442

443 **S3 Fig. Box plot showing the performance of a directly trained ANN and PLS binary**
444 **classifiers when applied to estimate the age classes of *An. gambiae* and *An. arabiensis* in the test**
445 **data set. (TIFF)**

446

447 **S4 Fig. Error distribution per actual age class of *An. gambiae* and *An. arabiensis* when directly**
448 **trained ANN and PLS binary classifiers applied to estimate age classes of mosquitoes in**
449 **training and test data sets, showing uniform distribution of errors (un-biased estimating)**
450 **across actual age classes of mosquitoes for ANN binary classifiers and un-uniform (biased**
451 **estimating) distribution for PLS classifiers. (TIFF)**

452

453 **S1 Table: Comparison of accuracies between directly trained binary classifiers and regressors**
454 **interpreted as binary classifiers. Results from ten-fold Monte Carlo cross-validation (TIFF**
455 **and DOCX)**

456

457 **S1 Appendix. Excel file with the *Anopheles gambiae* spectra used in the analysis. Column**
458 **header, wavelengths in 'nm'. (XLSX)**

459

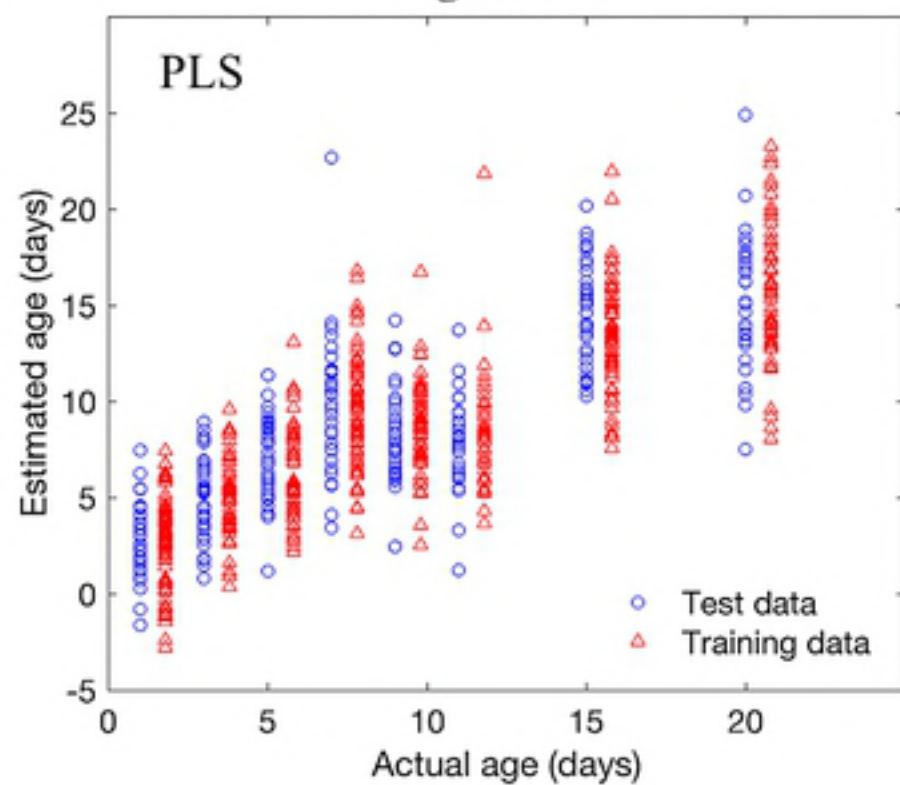
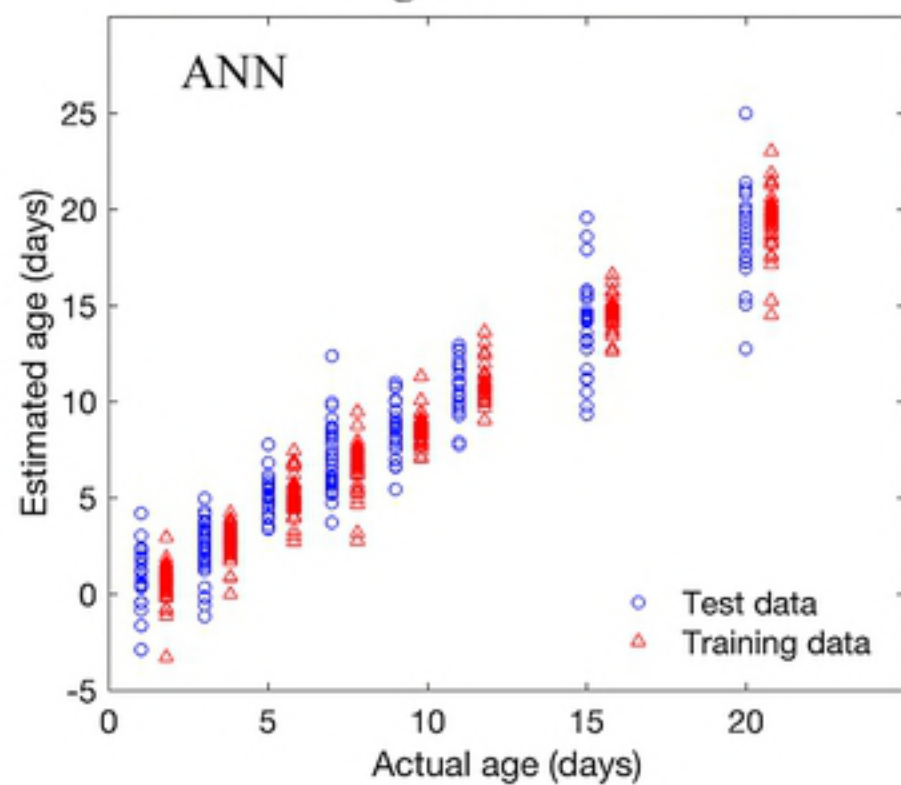
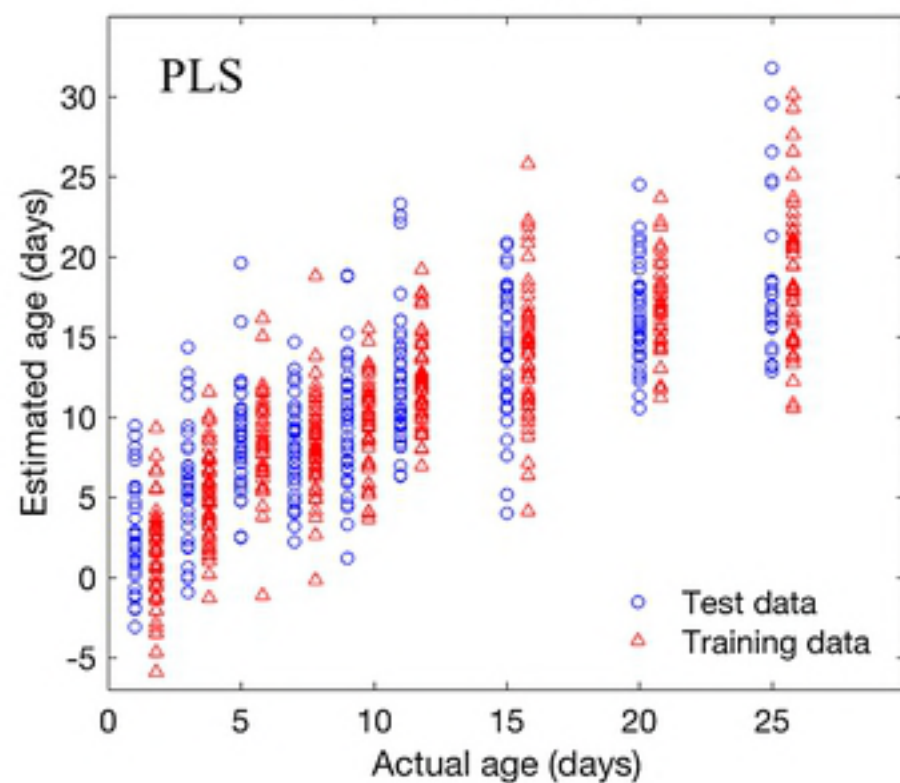
460 **S2 Appendix. Excel file with the *Anopheles arabiensis* spectra used in the analysis. Column**
461 **header, wavelengths in 'nm'. (XLSX)**

462

463 **S3 Appendix. Matlab code used to run the analysis for *Anopheles gambiae*. (M)**

464

465 **S4 Appendix. Matlab code used to run the analysis for *Anopheles arabiensis*. (M)**

A *An. gambiae***B** *An. gambiae***C** *An. arabiensis***D** *An. arabiensis*