Instar determination by constrained gaussian mixture models according to Dyar's rule

Abstract

In this study, gaussian mixture models with constrained parameter spaces are applied to the instar determination of insect species. Finite mixture models are often utilized to classify instars without knowing the instar number. Generally, parsimonious models with fewer free parameters would allow a more efficient estimation if underlying assumptions are valid. This work derives parsimonious gaussian mixture models using a parameter constraint motivated by Dyar's rule. Dyar's rule explains the growth pattern of larvae and nymphs of insects by assuming a constant ratio of head capsule width for every adjacent two development stages. Accordingly, every mean value of log-transformed data in each instar stage is considered a linear function. Two Dyar constants are an intercept and the slope of an instar stage, respectively, in the function. This idea allows estimating two free parameters instead of mean parameters in all instar stages in inferring the instar distribution. Along with this, the homogeneity of variance for every instar can be considered. As a result, four model hypotheses are proposed for each assumption of instar counts depending on whether or not these two parameter constraints are applied. After estimating models, the proposed method uses the ICL criterion to choose the optimal instar inference of models. Then, parametric bootstrap LR tests are applied to decide the most efficient model for parameter constraints given an instar counts inference. The proposed method represents that it can find the correct model settings during the simulation study, where various cases regarding mean parameters and the overlap between two groups are underlain. The result for *Meimuna mongolica* data shows that all observations are well divided into five instar stages. Parametric bootstrap LR tests also indicate the discrepancy of Dyar's rule in their datasets by selecting a model that does not assume Dyar's rule.

Keywords: Parsimonious models, model-based clustering, delineation of developmental stage, parametric bootstraps, statistical hypothesis testing

1 Introduction

Understanding the life cycle dynamics in an insect population is a significant issue in several areas of fundamental research and applied ecology, e.g., predicting the outbreak of pest insects. Precise information on the development stages of an insect species may be required in these fields. Due to an exoskeleton that limits growth, young insects grow step-wisely by their exuviation. Their life stages can be demarcated into several instar stages. However, for many species, the change of body size in successive two instar stages is often subtle or involves an intraspecies variation, so determining their instars is challenging. Accordingly, various clustering methods have been suggested to determine instars for samples of an insect species based on the size of morphological characters (Logan et al. 1998, Wu et al. 2013, Merville et al. 2014, Cen et al. 2018, Yang et al. 2018, Nguyen et al. 2022, Wang et al. 2022).

While some analyses for instar determination use model-free clustering methods (Yang et al. 2018, Nguyen et al. 2022), others use model-based clustering methods (Logan et al. 1998, Wu et al. 2013, Merville et al. 2014, Cen et al. 2018). One may consider a distributional assumption for each group. Finite mixture models are widely used for model-based clustering methods that assume every observation is randomly sampled from a heterogeneous population possessing several subgroups. In other words, samples can be divided into several groups, each following a different distribution. If all groups in a mixture distribution follow a normal distribution, it would be a gaussian mixture model. Let G be the number of groups or the number of mixture components. When it comes to univariate data, the gaussian mixture density can be written as

$$f(x) = \sum_{k=1}^{G} p_k \phi_k(x) \tag{1}$$

where p_k is the mixing proportion of kth group, $\sum_{k=1}^{G} p_k = 1,\ p_k > 0, \phi_k(x)$ is the normal density function of $N(\mu_k, \sigma_k^2)$ for $k = 1, \ldots, G$. Let $\boldsymbol{z} = (z_1, \ldots, z_G)$ be hidden group information of the sample. If $\boldsymbol{z}$ follows multinomial distribution with $(1; p_1, \ldots, p_G)$, i.e. $z_j = 1$ in a chance of p_j, while $z_k = 0$ for all $k \neq j$, the joint density of $(x, \boldsymbol{z})$ would be

$$f(x, \boldsymbol{z}) = \prod_{k=1}^{G} \Big(p_k \phi_k(x) \Big)^{z_k} \tag{2}$$

Unlike x, $\boldsymbol{z}$ and G are usually unknown and should be estimated. The expectation maximization (EM) algorithm defined in Dempster et al. (1977) is widely used to estimate $\boldsymbol{z}$ and the maximum likelihood (ML) estimate of model parameters in finite mixture models for a specified G. The advantage of using distributional assumptions is that it allows a solid statistical inference based on the decision theory. The mixture models can deal with classification issues in the overlapped area between two clusters with the Bayesian inference that interprets the group information of each observation as the posterior probability of belonging to a particular group given a data set (McLachlan & Peel 2000, Section 1.15). Regarding the decision theory, the highest posterior probability can be selected to determine the group for each observation. Besides, under the IID assumption—each sample is a random variable with an identical and independent mixture distribution to the other, the models allow the prediction of group information for future observations. However, the maximum likelihood estimation for gaussian mixture models often involves issues of a singularity or a spurious maximum when any pairs of sample points are sufficiently close together (Day 1969). Model constraints can deal with such issues by allowing a global maximized likelihood function (Hathaway 1985). Some parameter constraints, like common variance for every group, allow for making parsimonious models by reducing free parameters. Parsimonious models are often desirable because they provide a concise interpretation and tend to infer more precisely in model estimation and prediction when underlain assumptions are

valid.

Dyar's rule is a growth model of insects based on the assumption that indicates a regular geometric increase of head capsule width in consecutive instar stages (Dyar 1890). The model can be derived by

$$y = a\mathrm{e}^{b(k-1)} \tag{3}$$

where y is the size of a morphological character, a, b are Dyar's constants, and k indicates an instar stage. It implies the progression of instars will multiply e^b in the size of a morphological trait. Equation (3) is equivalent to

$$\log y = \log a + b(k-1) \tag{4}$$

It indicates that the average size of insects in an instar can be explained by a function of Dyar constants and their developmental stage. Accordingly, conformity to Dyar's rule can provide information on growth patterns in an insect species. Many studies about instar determination for an insect species evaluate the conformality of Dyar's rule to their models (Logan et al. 1998, Wu et al. 2013, Sukovata 2019, Peterson et al. 2019, Nguyen et al. 2022). If the result indicates nonconformity to Dyar's rule, it can be interpreted as underestimating or overestimating the number of instars (Sukovata 2019), or the violation of Dyar's rule in its growth (Peterson et al. 2019).

This study suggests parsimonious gaussian mixture models for instar delineation with parameter constraints by Dyar's rule. First, the log-transformed morphology data is assumed to follow a mixture of normal distributions. While the normality assumption of raw morphological data is frequently used in previous studies (Logan et al. 1998, Wu et al. 2013, Merville et al. 2014, Peterson et al. 2019), the log normality assumption can be realistic in practice when it comes to the length measurement because the length cannot be extended to the below zero. It often involves the right-skewed distribution whose skewness

can be alleviated by log transformation. Additionally, the mean parameter of every mixture component is regarded as a linear function of Dyar's constants and the developmental stages, just like equation (4). This assumption is likely valid when the average growth of a morphological trait in any two successive instars follows Dyar's rule. Consequently, it allows parsimonious gaussian mixture models by replacing mean parameters with a function of two free parameters for Dyar constants. The homogeneous variance assumption for all groups also reduces free parameters. Hence, four hypotheses are suggested according to Dyar's rule (hypothesis 1 & 2: hold; hypothesis 3 & 4: not hold) and variance homogeneity in different groups (hypothesis 1 & 3: hold; hypothesis 2 & 4: not hold) for each instar number assumption. Without the constraints over parameters, the most complex gaussian mixture model will be generated.

The gaussian mixture models can be used to determine the consistency of Dyar's rule for an insect species with statistical hypothesis testing. That is important because while some studies suggest Dyar's rule does not adapt to explain nymphal growth for some insect species (Peterson et al. 2019, Nguyen et al. 2022), many studies rely on a simple correlation coefficient between clustered labels and data or ratios between Dyar constants in subsequent instars (Merville et al. 2014, Sukovata 2019, Nguyen et al. 2022, Wang et al. 2022). These approaches may provide some information about instar stages but do not provide statistically powerful tests to make a decision. In contrast, the proposed method allows testing hypotheses of growth patterns in instar groups based on statistical inference if having a sufficient sample size. Suppose the test for an insect species suggests that models without Dyar's rule outperform those resting on Dyar's rule. It can be concluded that Dyar's rule assumption is unreliable in modeling its instar stages in such cases. Still, such a conclusion should rely on the normality assumption of log-transformed data. Thus, in case of the violation of the distributional assumptions will be discussed at the end of the

paper.

This article provides a procedure for estimating models, choosing the optimal instar counts, and testing the model hypotheses. A simulation study is presented in the result section on the performance of the proposed method. Finally, the proposed method is applied to determine the instar stages of a cicada species with a dataset collected by Hou et al. (2015).

2 Methods

Constrained gaussian mixture models

Models for cluster analysis are defined as follows. From equation (1), define a mixture component density as $\phi_k(x; \mu_k, \sigma_k^2)$ and a mixing proportion as p_k for $k = 1, \ldots, G$. Let a vectorized mean parameter of all mixture components be $\boldsymbol{\mu} = (\mu_1, \ldots, \mu_G)^T \in \mathrm{R}^G$ and that of their variance parameters be $\boldsymbol{\sigma^2} = (\sigma_1^2, \ldots, \sigma_G^2)^T \in (0, \infty)^G$. For hypotheses regarding Dyar's rule, $\boldsymbol{\mu}$ should be selected from $\{M\boldsymbol{\beta} : \boldsymbol{\beta} = (\beta_0, \beta_1)^T \in \mathrm{R}^2\}$, where M is defined as

$$M = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ \vdots & \vdots \\ 1 & G-1 \end{bmatrix} \tag{5}$$

Then, $\boldsymbol{\mu} = M\boldsymbol{\beta}$ implies $\mu_1 = \beta_0,\ \mu_2 = \beta_0 + \beta_1, \ldots,\ \mu_G = \beta_0 + (G-1)\beta_1$. For hypotheses regarding homogeneity in variance , $\sigma_1^2 = \sigma_2^2 = \cdots = \sigma_G^2$, where $\sigma_1^2 \in (0, \infty)$. Chauveau & Hunter (2013) devised an Expectation Conditional Maximization (ECM) algorithm for gaussian mixture models with linear parameter constraints to obtain ML estimates. Accordingly, EM and ECM algorithms are proposed based on Chauveau & Hunter (2013) to obtain model estimates regarding hypotheses 1–4 for each instar number assumption. Let,

n be the number of samples and $\boldsymbol{x} = (x_1, \ldots, x_n)$. Given $\boldsymbol{x}$ with the independent sample assumption, the EM and ECM algorithms will provide $\hat{\boldsymbol{z}}_i = (\hat{z}_{i1}, \ldots, \hat{z}_{iG})$, the posterior probability for hidden group information of ith sample given x_i. For the clustering analysis of x_i, the index $k \in \{1, \ldots, G\}$ that makes the largest $\hat{z}_{ik}$ will be chosen to determine its group. The supplementary material provides details of the proposed EM and ECM algorithms.

Instars number selection

To infer reasonable instar stages, suggested instar counts G are confined to a specific range while estimating models. After the estimation procedure, the optimal number of instars for each parameter constraint hypothesis will be chosen by minimizing the Integrated Classification Likelihood (ICL) derived by Biernacki et al. (2000) using the Bayesian information criterion (BIC) (Schwarz 1978). BIC and ICL are represented as equations (6) and (7).

$$\text{BIC} = -2\sum_{i=1}^{n} \log f(x_i; \hat{\Theta}) + q \log n \tag{6}$$

where q is the number of free parameters defined as

$$q = G - 1 + \begin{cases} 2, & \text{Dyar's rule} \\ G, & \text{non-Dyar's rule} \end{cases} + \begin{cases} 1, & \text{variance homogeneity} \\ G, & \text{variance heterogeneity} \end{cases}.$$

$$\text{ICL} = \text{BIC} - 2\sum_{i=1}^{n}\sum_{k=1}^{G} \hat{z}_{ik}^{MAP} \log \hat{z}_{ik} \tag{7}$$

$$\text{where } \hat{z}_{ik}^{MAP} = \begin{cases} 1, & \hat{z}_{ik} = \max_{1 \le j \le G} \hat{z}_{ij} \\ 0, & \text{otherwise} \end{cases} \quad \text{for each } i = 1, \ldots, n;\ k = 1, \ldots, G$$

$\hat{\Theta}$ is the ML estimate of parameters given a model hypothesis, and $\hat{z}_{ik}^{MAP}$ is a maximum a posteriori probability (MAP) estimate of group information for i, k. Suppose selected instar

numbers are different for each hypothesis. In that case, the final decision for the instar number can be the number held by the hypothesis with minimal ICL or in a subjective manner by interpreting the results.

Parametric bootstrap LR tests

Statistical model assumptions enable to test of model hypotheses regarding parameter constraints. Let $\mathcal{M}_G$ be a family of $G-$component gaussian mixture models regarding hypotheses 1–4, $M_{lG} \in \mathcal{M}_G$ be a model for hypothesis $l,\ l = 1, 2, 3, 4$. To compare hypotheses 2–4 to hypothesis 1, one may consider the likelihood ratio (LR) test by calculating the maximum LR statistics given the null and alternative hypotheses. For each $M_{iG} \in \mathcal{M}_G / M_{1G}$

$$H_0 : \text{observations arise from } M_{iG} \quad \text{versus} \quad H_1 : \text{observations arise from } M_{1G}$$

$$\mathrm{LR}_{iG} = -2\Big(L(\hat{\Theta}_{iG}) - L(\hat{\Theta}_{1G})\Big) \tag{8}$$

where $L(\Theta) = \log f(\boldsymbol{x}; \Theta)$ is the log-likelihood function and Θ_{lG} are a collection of model parameters of M_{lG}, for each $l = 1, 2, 3, 4$ and $\hat{\Theta}_{lG}$ is the ML estimator of Θ_{lG}, and LR_{iG} is the consequent LR statistic. The null distribution of LR_{iG} is usually assumed to be asymptotic χ^2-distributed with $q_{\mathrm{LR}} = q_{1G} - q_{iG}$ degrees of freedom, where q_{1G} and q_{iG} denote the number of free parameters for M_{1G} and M_{iG}, respectively. However, since mixture models do not guarantee the regularity condition of ML estimators, the null χ^2-distribution does not provide a valid approximation for the LR statistic (McLachlan & Peel 2000, Section 6.4). As a result, conventional LR tests can lead to an inaccurate decision in comparing hypotheses, even with large sample size. A resampling method is an alternative approach to this (McLachlan 1987, McLachlan & Peel 2000, Section 6.6, Punzo et al. 2016). The parametric bootstrap LR test generates LR_{iG} replications as follows. n bootstrap samples are generated from the model M_{iG} under H_0, where Θ_{iG} is replaced by its ML estimate

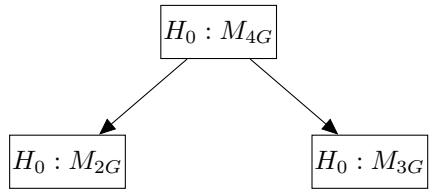

Figure 1: Graph of hierarchical relationships between the null hypotheses

calculated under H_0 from the original dataset. The value of LR_{iG} replication is computed by fitting models M_{iG} and M_{1G} with the bootstrap samples. This process is independently repeated R times, and the R replicated values of LR_{iG} can approximate a null sampling distribution for LR_{iG} as R is increased. Consequently, a test can reject H_0 when LR_{iG} for the original data overlies the specified critical region in the approximated null distribution of LR_{iG}.

Since this process is computationally intensive when R is large, without an interest in estimating a precise p-value, the following criterion allows using a moderate number of R. Aitkin et al. (1981) note that the bootstrap replications can provide a test of approximate α, where α is a specified significance level for the test. If LR_{iG} for the original data is greater than the hth smallest of its R bootstrap replications, the test that rejects H_0 has an approximate size of α as

$$\alpha = 1 - \frac{h}{R+1} \tag{9}$$

According to equation (9), for a specific α, the value of R and h can be chosen. For instance, when $\alpha = 0.05$, $R = 99$ with $h = 95$ can be used to approximate α. Although this hypothesis testing can be applied to assess the number of G as well (McLachlan 1987), I suggest a procedure that chooses G with the ICL criterion and then tests hypotheses 1–4 with the parametric bootstrapping method to reduce its computation requirement. If a

$H_0:$	M_{4G}	M_{2G}	M_{3G}		Decision
	NR	-	-	$\Longrightarrow$	M_{4G}
	R	NR	NR	$\Longrightarrow$	M_{4G}
	R	NR	R	$\Longrightarrow$	M_{2G}
	R	R	NR	$\Longrightarrow$	M_{3G}
	R	R	R	$\Longrightarrow$	M_{1G}

NR: not rejecting H_0

R: rejecting H_0

Table 1: The decision for testing $H_0 : M_{iG},\ i = 2, 3, 4$ vs. $H_1 : M_{1G}$ given an instar count G

hypothesis involves a nested model of the other hypothesis, this forms a hierarchy between the null hypotheses (Figure 1). For instance, M_{2G} includes M_{4G}, and this means M_{4G} is more restricted than the former. Hence, $H_0 : M_{4G}$ should be tested at first when deciding on a final model because its rejection is a necessary condition for the rejection of other hypotheses. As long as M_{4G} is rejected, $H_0 : M_{2G}$ and $H_0 : M_{3G}$ are tested, otherwise M_{4G} is accepted. Two tests for M_{2G} and M_{3G} can decide the conformity to Dyar's rule and variance homogeneity in model hypotheses. For example, if $H_0 : M_{2G}$ is rejected while $H_0 : M_{3G}$ not rejected, the final model decision is M_{3G}. Table 1 represents a way to determine the final model by consecutive tests given G. Since multiple testing of M_{2G} and M_{3G} can increase the Type 1 error in its decision, α for their tests should be controlled by the familywise error rate. For instance, apply $\alpha/2$ as a significance level for tests of M_{2G} and M_{3G} by the Bonferroni correction.

All the procedure is programmed with `R` software (R version 4.2.1). For the EM and ECM algorithm procedure, `R` package `mixtools` (Tatiana et al. 2009) can be applied in mixture models with a linear parameter constraint, but I use customized code to adapt the proposed method.

Simulation study

A simulation study with various situations is conducted to show the performance of the proposed procedure to find an optimal instar inference. To assume specific cases in which a sampling distribution has an overlapped region between adjacent instar groups, I adopt the normalized overlap measure used by Punzo et al. (2016). The set of overlap measures is defined by $B = \{B_{k,k-1} : k = 2, \ldots, G\}$, where $B_{k,k-1}$ is the measure of overlap between kth and $(k-1)$th groups based on Bhattacharyya (1943) with a definition

$$B_{k,k-1} = \exp\left(-\frac{1}{4(\sigma_k^2 + \sigma_{k-1}^2)}(\mu_k - \mu_{k-1})^2 - \frac{1}{2}\log\left(\frac{1}{2}\sqrt{\sigma_k^2 + \sigma_{k-1}^2}\right)\right)$$

Since every $B_{k,k-1}$ is laid between 0 and 1, $\max B = 0$ implies the absence of overlapped region, whereas $\max B = 1$ implies at least two adjacent groups are completely overlapped in a population. I consider twelve cases of $n = 500$ with various conditions regarding Dyar's rule, variance homogeneity, overlapping between groups, and mixing proportions. For cases 1 and 3, Dyar's rule and variance homogeneity are assumed, whereas incongruous Dyar's rule and common variance are assumed for cases 2 and 4. Cases 1–4 are assumed to be a uniform mixture of different groups. For cases 5 and 6, groups with an unbalanced mixing proportion, Dyar's rule, and variance homogeneity are assumed. Their mixing proportion imitates a proportion of the first to the sixth instars regarding *Blattella asahinai* data collected by Peterson et al. (2019). Cases 7–12 are variance heterogeneity versions of cases 1–6. The details of the twelve cases are noted in Table 2. One hundred datasets for each case are generated to conduct the simulation study. Figure 2 represents the theoretical distributions of twelve cases described in Table 2, where simulation data was generated.

Real data

For the application, head capsule width measurements for juveniles of insect species were collected from the literature. Hou et al. (2015) studied instar stages for *Meimuna*

No.	G	μ^{a}	$\sigma^{2\,\mathrm{b}}$	B^{c}	Mixing proportions^{d}	Model
1	5	$-1/-0.25/0.5/1.25/2$	all 0.01	< 0.01	all 0.2	M_{4G}
2		$-1/-0.1/0.8/1.55/2.3$	all 0.01	< 0.01	all 0.2	M_{2G}
3		$-1/-0.25/0.5/1.25/2$	all 0.2875	0.25	all 0.2	M_{4G}
4		$-1/-0.1/0.8/1.55/2.3$	all 0.2875	0.25	all 0.2	M_{2G}
5	6	$-1/-0.25/0.5/1.25/2/2.75$	all 0.01	< 0.01	0.133/0.227/0.217/0.2/0.131/0.092	M_{4G}
6		$-1/-0.25/0.5/1.25/2/2.75$	all 0.02875	0.25	0.133/0.227/0.217/0.2/0.131/0.092	M_{4G}
7	5	$-1/-0.25/0.5/1.25/2$	0.006/0.007/0.008/0.009/0.01	< 0.01	all 0.2	M_{3G}
8		$-1/-0.1/0.8/1.55/2.3$	0.006/0.007/0.008/0.009/0.01	< 0.01	all 0.2	M_{1G}
9		$-1/-0.25/0.5/1.25/2$	0.015/0.0189/0.0227/0.0266/0.0305	$0.05-0.25$	all 0.2	M_{3G}
10		$-1/-0.1/0.8/1.55/2.3$	0.015/0.0189/0.0227/0.0266/0.0305	$0.01-0.25$	all 0.2	M_{1G}
11	6	$-1/-0.25/0.5/1.25/2/2.75$	0.006/0.007/0.008/0.009/0.01/0.011	< 0.01	0.133/0.227/0.217/0.2/0.131/0.092	M_{3G}
12		$-1/-0.25/0.5/1.25/2/2.75$	0.015/0.018/0.021/0.024/0.027/0.03	$0.05-0.25$	0.133/0.227/0.217/0.2/0.131/0.092	M_{3G}

a the mean parameter for each group

b the variance parameter for each group

c the range of $B_{k,k-1}$ in B

d the mixing proportion for each group

Table 2: Twelve simulation settings regarding Dyar's rule, variance homogeneity, overlapping between groups, and mixing proportion

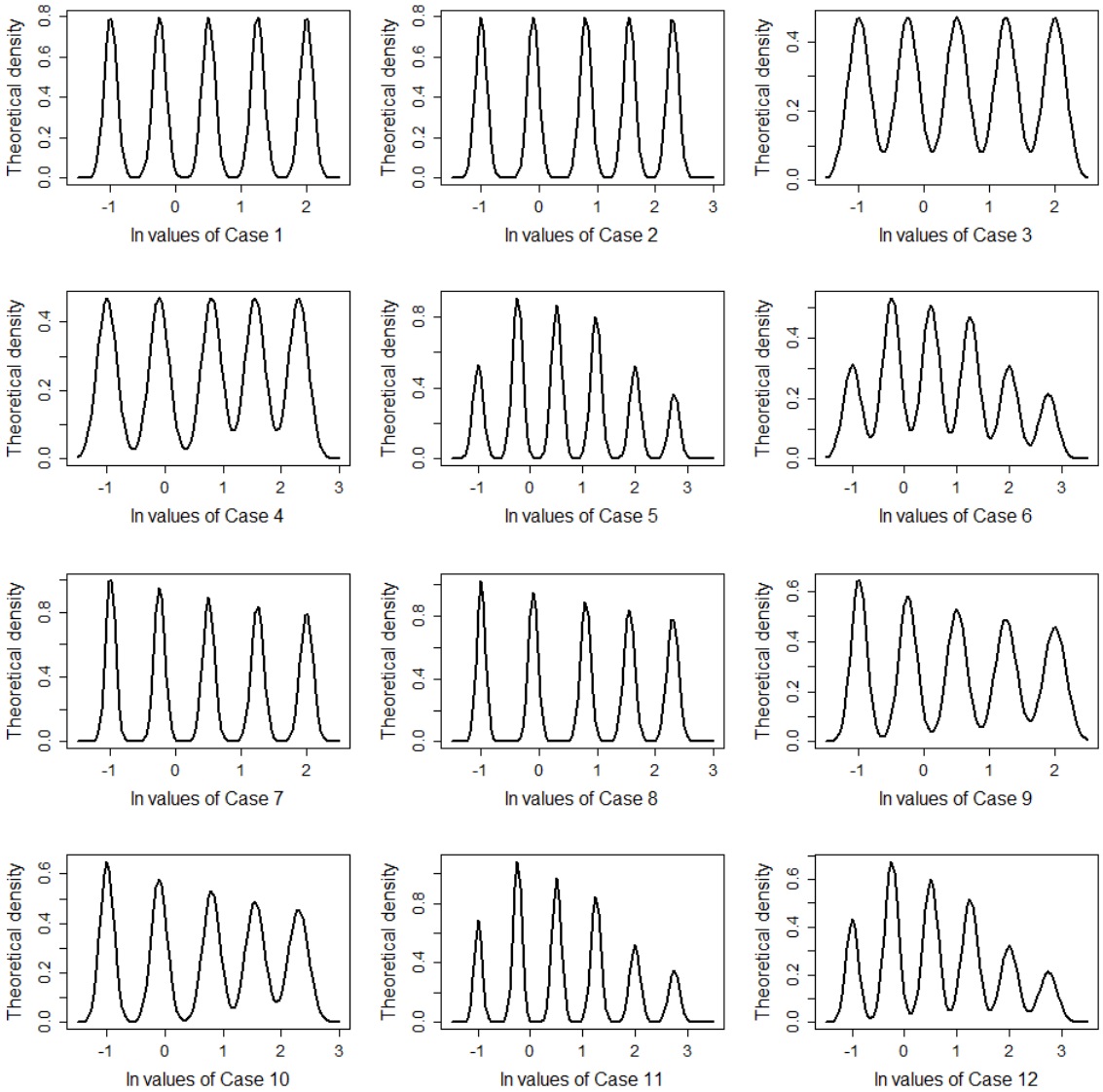

Figure 2: The theoretical distribution for log-transformed (ln) samples regarding cases 1–12

mongolica nymphs. They collected their samples from a laboratory and fieldwork. The first instar nymphs were raised in the laboratory, while older nymphs were excavated in the ground by fieldwork. They took pictures of the samples and measured the length of morphological characters, including head capsule and abdominal width, by Image Lab version 2.2.4.0 (resolution: 0.01 mm). Hou et al. (2015) suggested five instar stages of *M. mongolica* because according to the head capsule measurement, their samples were perfectly separated into five groups with naked eyes with no overlapping. However, this information is ignored while estimating constrained gaussian models. All the raw data were log-transformed before entering the model estimation. Due to the repeated values in datasets, the EM algorithm likely involves the singularity issue when G is large. Since the measurement depends on a resolution of 0.01 mm, some observations possibly have the same value, although their variable type is continuous. In dealing with such issues, raw data can be added to random noise generated with $U_i \sim \text{Unif}(-0.005, 0.005)$mm.

3 Results

Simulation study

The simulation study of the twelve cases was implemented in `R`. The data analysis for each case was repeated with one hundred datasets. Tables 3 and 4 represent the results of searching the optimal instar counts and model hypotheses regarding Dyar's rule and variance homogeneity. Grey highlighted numbers imply positions of the actual model setting for each case. The ICL criterion searched for correct instar stages in 99 to 100 percent of the datasets for all cases (Table 3). However, it did not pinpoint the correct model settings for cases 7–12 that assume heterogeneous variance because it tended to select models assuming variance homogeneity. I considered the ICL criterion found a correct inference

	Inference of instar counts[a]						Model minimizing ICL[b]			
Case No.	3	4	5	6	7	8	M_{1G}	M_{2G}	M_{3G}	M_{4G}
1	0	0	99[c]	1	0	0	0	0	0	100[d]
2	0	0	99	1	0	0	0	100	0	0
3	0	0	100	0	0	0	0	0	0	100
4	0	0	99	1	0	0	0	100	0	0
5	0	0	0	100	0	0	0	0	0	100
6	0	0	0	100	0	0	0	0	0	100
7	0	0	99	1	0	0	0	0	1	99
8	0	0	99	1	0	0	0	100	0	0
9	0	0	99	1	0	0	0	0	11	89
10	0	0	99	1	0	0	9	91	0	0
11	0	0	0	100	0	0	0	0	3	97
12	0	0	0	100	0	0	0	0	2	98

[a] instar count inference by a model that minimizes ICL in whole hypotheses

[b] a model minimizes ICL

[c] the position of grey-highlights implies the true instar number in each case

[d] the position of grey-highlights implies the true model in each case

Numbers in the table indicate percentages of a section for each case that refer to 100 datasets

Table 3: Model decision of parametric LR tests given an instar count G for the simulation study

Case No.	α[a]	G	M_{1G}	M_{2G}	M_{3G}	M_{4G}	$M_{1G} + M_{2G}$[b]	$M_{3G} + M_{4G}$[c]
1	0.05	5	0	1	3	96[d]	1	99
2			4	96	0	0	100	0
3			0	1	3	96	1	99
4			5	95	0	0	100	0
5		6	1	1	2	96	2	98
6			2	1	1	96	3	97
7		5	1	0	45	54	1	99
8			55	45	0	0	100	0
9			2	0	68	30	2	98
10			75	25	0	0	100	0
11		6	0	0	44	56	0	100
12			2	0	45	53	2	98
1	0.10	5	0	2	5	93	2	98
2			5	95	0	0	100	0
3			0	2	5	93	2	98
4			6	94	0	0	100	0
5		6	1	2	4	93	3	97
6			3	1	3	93	4	96
7		5	2	0	58	40	2	98
8			66	34	0	0	100	0
9			6	0	71	23	6	94
10			82	18	0	0	100	0
11		6	0	1	52	47	1	99
12			4	0	54	42	4	96

[a] the familywise error rate of multiple testing

[b] the sum of counts for models nonconforming to Dyar's rule

[c] the sum of counts for models conforming to Dyar's rule

[d] the position of grey-highlights implies the true model in each case

Numbers in the table indicate percentages for each case that refer to 100 datasets

Table 4: Model decision of parametric LR tests given an instar count G for the simulation study with 99 bootstrap replications

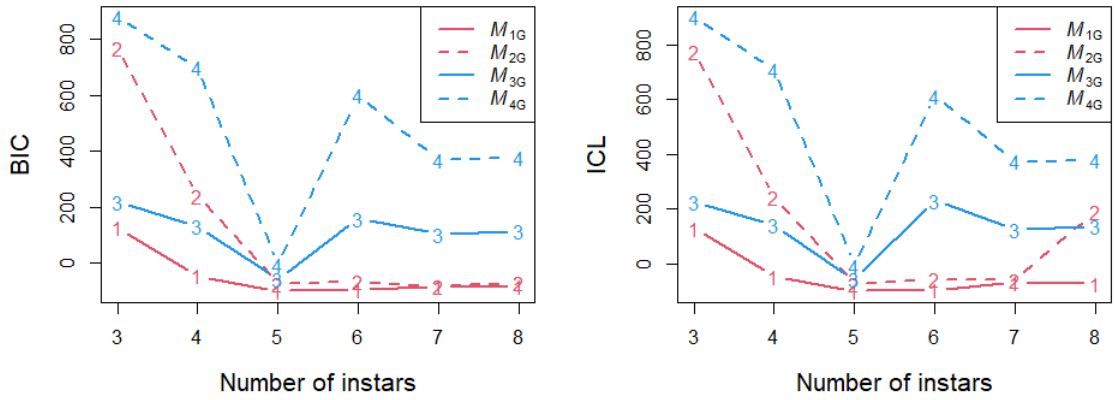

Figure 3: The comparison of BIC and ICL for hypothesis 1–4 of *M. mongolica* data; numbers in the plot indicate model hypothesis indices

for instar counts, and then conducted parametric bootstrap LR tests under the correct number of instars with 99 bootstrap replications (Table 4). They selected around 95 percent of correct models assuming variance homogeneity regardless of Dyar's rule conformity, overlapped groups, and balance in mixing proportion for cases 1–6. They also found the correct setting of around half a hundred for cases 7–12. Regarding only being interested in Dyar's rule consistency, the tests searched 94 to 100 percent of the correct assumption in all cases. The familywise error of 0.1 slightly increased the power of tests in cases 7–12 compared to that of 0.05 but also increased errors in Dyar's rule conformity.

Data analysis for *Meimuna mongolica*

I compared hypotheses 1–4 with instar counts $G = 3, \ldots, 8$ for head-capsule data of *M. mongolica*. According to the ICL criterion, the five instars assumption for *M. mongolica* was held for all the hypotheses (Figure 3). Although more parsimonious models had fewer

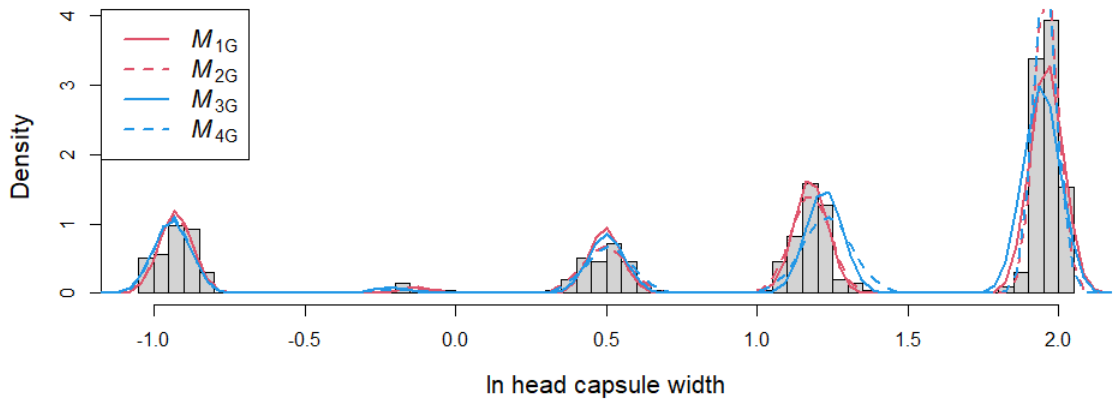

Figure 4: The histogram (n=391) and estimated probability density for log-transformed head capsule data of *M. mongolica* assuming five instar stages

Model	q^{a}	BIC	ICL	LR_{i5}	$q_{\mathrm{LR}}{}^{\mathrm{b}}$	p-value^{c}
M_{45}	7	-6.38	-6.38	135.0	7	0.0112
M_{35}	11	-57.92	-57.92	59.5	3	0.0092
M_{25}	10	-71.70	-71.70	51.7	4	0.0010
M_{15}	14	-99.55	-99.55			

a the number of free parameters for each model

b degrees of freedom of LR_{i5} statistics

c bootstrap p-value of LR_{i5} statistics under M_{i5}

Table 5: Details on the parametric LR test assuming five instar stages to the *M. mongolica* data with 999 bootstrap replications

Instar	Count	Mean ± Std[a](mm)	Range (mm)	Dyar constant
1	64	0.40 ± 0.02	0.36–0.43	-
2	5	0.88 ± 0.06	0.84–0.99	2.20
3	51	1.65 ± 0.13	1.38–1.92	1.88
4	90	3.25 ± 0.21	2.82–3.86	1.97
5	181	7.10 ± 0.28	6.10–7.68	2.18

a sample standard deviation for each instar group

Table 6: The optimal inference for *M. mongolica* from the proposed procedure using log-transformed head capsule data

free parameters that penalize BIC, the most complex one showed better performance in terms of ICL. Since models were well fitted with data, ICL was not significantly deviated from BIC and gave almost the same value at $G = 5$ (Figure 3 and Table 5). The estimated density of five instars for all hypotheses showed a penta-modal distribution though the second (left to right) modal had a relatively low height (Figure 4). Models that assume Dyar's rule (M_{3G} & M_{4G}) were slightly mismatched at the fourth modal of the histogram, whereas those that do not assume Dyar's rule (M_{1G} & M_{2G}) were more congruent to the histogram. The parametric bootstrap LR tests were conducted under the five-instar assumption with 999 bootstrap replications. According to the test procedure, M_{15} was selected with a familywise error rate of 0.05 (Table 5). As a result, it can be concluded that models without assuming Dyar's rule and homogeneous variance deliminate the instar stages of *M. mongolica* well. The clustering analysis provided the exact same result for all models assuming five instar stages (Table 6).

4 Discussion

This study aims to make an efficient clustering procedure that determines the instar stage of insect samples by using model parsimony. Before using the proposed method, confirmation for collecting sufficient samples in every instar stage is desirable. Gaussian mixture models require sufficient samples to obtain precise estimates for their parameters. For each group of instar stages, at least two samples with different values are needed to avoid a singularity issue in calculating maximum likelihood estimates. Otherwise, the model procedure may not work well or lead to an erroneous outcome. Heterogeneous variances of groups can entail many local maxima in the likelihood function (Basford & McLachlan 1985) and make a spurious solution during the model estimation. Therefore, choosing an appropriate initial value for estimating models is essential to obtaining an intended result. The K-means clustering algorithm is one way to obtain a proper initial value for the ECM and EM algorithms. Models restricted by Dyar's rule have an advantage in avoiding these overfitting issues compared to unconstrained models. For each group, the estimated mean parameters are hard to match a single data point because a vector for mean parameters derived from all groups should be projected in the column space of its linear constraint during the estimation process. Accordingly, they can prevent overfitting problems that fall into unintended solutions caused by a few data points. If data conforms to Dyar's rule, they may give a more precise inference for instar stages because they use all samples to estimate the structure of a growth pattern in an insect species.

The data analysis of *M. mongolica* shows that the dataset does not match Dyar's rule according to the parametric bootstrap tests under gaussian mixture model assumptions. Then, it shows possible discrepancies to Dyar's rule in *M. mongolica*'s growth and gaussian mixture models without the parameter constraints seem to perform better in its instar

classification. Before interpreting the result, issues involved in sampling and other issues regarding distributional assumptions should be considered. Datasets of Hou et al. (2015) and Nguyen et al. (2022) may include heterogeneous observations. First, their observations are not the IID samples. They integrated data for the first nymphs raised in a laboratory into fieldwork data, leading to heterogeneous sampling. Besides, the fieldwork process in their study can generate a sampling bias. Except for the laboratory data, only a few small-sized cicada observations considered the second-stage instars were sampled in the fieldwork. This may indicate their methods did not adapt to collecting small-sized cicada nymphs. Therefore, more observations will be needed to evaluate Dyar's rule in its growth and improve the accuracy of instar determination for the dataset.

While a log-normal distribution for each group is assumed in this study, violating the normality assumption can entail an inaccurate classification. Unfortunately, there is no complete method to evaluate the validity of the distributional assumption. The histogram of clustering analysis results can be used to check whether each mixture component follows a bell-shaped distribution. The log transformation possibly does not guarantee the validity of the normality assumption. Many instar determinations studies assume a normal distribution of observations for each instar stage (Logan et al. 1998, Wu et al. 2013, Merville et al. 2014), whereas the result of Peterson et al. (2019) showed that gaussian mixture models using log-transformed data provided more discrete clusters than those using untransformed data. If the log-transformation does not lead to a bell-shaped distribution for each mixture component or ambiguous clustering results, another distributional assumption can be considered for such data sets. An alternative approach for this issue is assuming a mixture density for each instar stage. Hou et al. (2015) suggested a morphological variation in *M. mongolica* that belong to the third and fourth instar stages. The difference in food availability for each place may affect their growth. The variation among habitats can affect the

population's stability, the degree of interspecific competition, and the fitness for characteristics, which may lead to morphological diversity. For some species, sexual dimorphism may contribute to the discrepancy in the data homogeneity (Turner & Williams 2005). A mixture distribution for each instar stage will represent this heterogeneity of data. Consequently, a mixture model with two or more normal mixture components instead of normal densities can be applied in the proposed procedure.

The proposed method provides an efficient way to determine instar stages under appropriate distributional assumptions. Further study is required to examine nymphal growth for various insect species and the peculiarity of the size distribution of nymphs and larvae. Accordingly, future work will investigate to relieve assumptions for adapting general cases having different distributional shapes.

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SUPPLEMENTARY MATERIAL

Expectation Conditional Maximization (ECM) algorithm

Let $\nu_k = 1/\sigma_k^2$ for $k = 1, \ldots, G$, $\boldsymbol{\nu} = (\nu_1, \ldots, \nu_G)$

Input: $\boldsymbol{x}, G, \boldsymbol{p}^{(0)} = (p_1^{(0)}, \ldots, p_G^{(0)}), \boldsymbol{\mu}^{(0)} = (\mu_1^{(0)}, \ldots, \mu_G^{(0)}), \boldsymbol{\nu}^{(0)} = (\nu_1^{(0)}, \ldots, \nu_G^{(0)}), \epsilon$

Output : $\left(\hat{z}_{ik}\right)_{1 \le i \le n; 1 \le k \le G}, \hat{\boldsymbol{p}}, \hat{\boldsymbol{\mu}}, \hat{\boldsymbol{\nu}}$

Repeat: for $t = 1, \ldots, 5000$

E-step: Compute $\hat{z}_{ik}^{(t)} = \frac{p_k^{(t-1)} \phi_k(x_i)^{(t-1)}}{\sum_j^G p_j^{(t-1)} \phi_j(x_i)^{(t-1)}}$ for each i, k

M-step: Update $\boldsymbol{p}, \boldsymbol{\mu}, \boldsymbol{\nu}$ by

$$\boldsymbol{p}^{(t)} = \left(\frac{\sum_{i=1}^n \hat{z}_{ik}^{(t)}}{n} \right)_{k=1,\ldots,G} \tag{10}$$

$$\boldsymbol{\beta}^{(t)} = \left(M^T D(\boldsymbol{\nu}_{Zt}) M \right)^{-1} M^T D(\boldsymbol{\nu}^{(t-1)}) Z_t^T \boldsymbol{x} \tag{11}$$

where $M = I_G$ for hypotheses 1 & 2, equation (5) for hypotheses 3 & 4.

$$D(\boldsymbol{\nu}) = \begin{cases} \nu_k, & \text{kth diagonal element} \\ 0, & \text{otherwise} \end{cases} \in \mathrm{R}^{G \times G}$$

$$Z_t = \left(\hat{z}_{ik}^{(t)} \right)_{1 \le i \le n;\ 1 \le k \le G} \in \mathrm{R}^{n \times G}, \ \boldsymbol{x} = (x_1, \ldots, x_n)^T \in \mathrm{R}^n$$

$$\boldsymbol{\nu}_{Zt} = \left(\nu_1^{(t-1)} \sum_{i=1}^n \hat{z}_{i1}^{(t)}, \nu_2^{(t-1)} \sum_{i=1}^n \hat{z}_{i2}^{(t)}, \ldots, \nu_G^{(t-1)} \sum_{i=1}^n \hat{z}_{iG}^{(t)} \right) \in \mathrm{R}^G.$$

$$\boldsymbol{\mu}^{(t)} = M \boldsymbol{\beta}^{(t)}$$

for $i = 1, \ldots, n, \quad k = 1, \ldots, G$

$$\tilde{z}_{ik}^{(t)} = \begin{cases} \frac{p_k^{(t)} \phi_k(x; \mu_k^{(t)}, \nu_k^{(t)})}{\sum_j^G p_j^{(t)} \phi_j(x; \mu_j^{(t)}, \nu_j^{(t)})}, & \text{hypothesis 3} \qquad \text{(extra E-step)} \\ \hat{z}_{ik}^{(t)}, & \text{hypothesis 1,2, \& 4} \end{cases} \tag{12}$$

$$\boldsymbol{\nu}^{(t)} = \begin{cases} \left(\frac{\sum_i^n \tilde{z}_{ik}^{(t)}}{\sum_i^n \tilde{z}_{ik}^{(t)} (x_i - \mu_k^{(t)})^2} \right)_{1 \le k \le G}, & \text{hypothesis 1 \& 3} \\ \frac{\sum_i^n \sum_{k=1}^G \tilde{z}_{ik}^{(t)}}{\sum_i^n \sum_{k=1}^G \tilde{z}_{ik}^{(t)} (x_i - \mu_k^{(t)})^2} 1_G, & \text{hypothesis 2 \& 4} \end{cases} \tag{13}$$

Until: converged in terms of $|\log f(\boldsymbol{x}; \boldsymbol{p}^{(t)}, \boldsymbol{\mu}^{(t)}, \boldsymbol{\nu}^{(t)}) - \log f(\boldsymbol{x}; \boldsymbol{p}^{(t-1)}, \boldsymbol{\mu}^{(t-1)}, \boldsymbol{\nu}^{(t-1)})| < \epsilon$

Initial value $\boldsymbol{p}^{(0)}, \boldsymbol{\mu}^{(0)}, \boldsymbol{\nu}^{(0)}$ are generated by a K-means clustering algorithm that divides observations into G groups.

In equation (11), all $\nu_k^{(t-1)}$ can be canceled out in $D(\boldsymbol{\nu}_{Z_t})$ and $D(\boldsymbol{\nu}^{(t-1)})$ for hypotheses 1,2, and 4, whereas $\nu_k^{(t-1)}$ are left for hypothesis 3. This precludes obtaining a closed-form solution if updating $\boldsymbol{p}, \boldsymbol{\mu}, \boldsymbol{\nu}$ simultaneously during the M-step of models for hypothesis 3. Consequently, their estimation requires the ECM algorithm that fixes $\boldsymbol{\nu} = \boldsymbol{\nu}^{(t-1)}$ when calculating $\boldsymbol{\beta}^{(t)}$ and imposes an extra E-step in equation (12) (Chauveau & Hunter 2013). In contrast, models for other hypotheses do not need such a process, and their algorithms are equivalent to the EM algorithm.

If an algorithm is terminated due to computational issues, like an occurrence of Not-A-Number (NaN), restart the algorithm with a different initial value.

The proof of E-step

Under the definition of equation (1) and the IID assumption, the probability mass function of $\boldsymbol{z}_i = (z_{i1}, \ldots, z_{iG})$ given $\boldsymbol{x}$ and $\Theta^{(t-1)} = (\boldsymbol{p}^{(t-1)}, \boldsymbol{\mu}^{(t-1)}, \boldsymbol{\nu}^{(t-1)})$ will be

$$f(\boldsymbol{z}_i|\boldsymbol{x}; \Theta^{(t-1)}) = f(\boldsymbol{z}_i|x_i; \Theta^{(t-1)}) = \prod_{k=1}^{G} \left(\frac{p_k^{(t-1)} \phi_k(x_i)^{(t-1)}}{\sum_j^G p_j^{(t-1)} \phi_j(x_i)^{(t-1)}} \right)^{z_{ik}}, \text{ then}$$

$$\hat{z}_{ik}^{(t)} = \mathrm{E}(z_{ik}|x_i, \Theta^{(t-1)}) = \frac{p_k^{(t-1)} \phi_k(x_i)^{(t-1)}}{\sum_j^G p_j^{(t-1)} \phi_j(x_i)^{(t-1)}}.$$

The proof of equations (10), (11), & (13) in M-step

Under equation (2), the complete log-likelihood is derived as

$$\begin{aligned} L_c(\boldsymbol{p}, \boldsymbol{\mu}, \boldsymbol{\nu}; \boldsymbol{x}, \boldsymbol{z}_1, \ldots, \boldsymbol{z}_n) &= \sum_{i=1}^{n} \log f(x_i, \boldsymbol{z}_i; \boldsymbol{p}, \boldsymbol{\mu}, \boldsymbol{\nu}) \\ &= \sum_{i=1}^{n} \sum_{k=1}^{G} z_{ik} \Big(\log p_k + \frac{1}{2} \log(\nu_k) - \frac{\nu_k}{2} (x_i - \mu_k)^2 \Big) + C \end{aligned}$$

where C is a constant.

Given $\boldsymbol{x}$ and $\Theta^{(t-1)}$, the expected L_c is defined as

$$
\begin{aligned}
Q(\boldsymbol{p}, \boldsymbol{\mu}, \boldsymbol{\nu}|\boldsymbol{x}, \Theta^{(t-1)}) &:= \mathrm{E}\Big(L_c|\boldsymbol{x}, \Theta^{(t-1)}\Big) \\
&\propto \sum_{i=1}^{n}\sum_{k=1}^{G} \hat{z}_{ik}\Big(\log p_k + \frac{1}{2}\log(\nu_k) - \frac{\nu_k}{2}(x_i - \mu_k)^2\Big)
\end{aligned}
$$

By the reparameterization of $\boldsymbol{p}$ with $\tilde{\boldsymbol{p}} = (p_1, \ldots, p_{G-1}),\ p_G = 1 - \sum_{j=1}^{G-1} p_j$

$$
\frac{\partial}{\partial \tilde{\boldsymbol{p}}} Q(\tilde{\boldsymbol{p}}|\boldsymbol{x}, \Theta^{(t-1)}) = \left(\sum_{i=1}^{n} \frac{\hat{z}_{ik}^{(t)}}{p_k} - \sum_{i=1}^{n} \frac{1 - \sum_{j=1}^{G-1} \hat{z}_{ij}^{(t)}}{1 - \sum_{j=1}^{G-1} p_j}\right)_{k=1,\ldots,G-1}
$$

$$
\frac{\partial}{\partial \tilde{\boldsymbol{p}}} Q(\tilde{\boldsymbol{p}}|\boldsymbol{x}, \Theta^{(t-1)})_{\tilde{\boldsymbol{p}}=\tilde{\boldsymbol{p}}^+} = 0,\ p_G^+ = 1 - \sum_{j=1}^{G-1} p_j^+ \implies p_k^+ = \frac{\sum_{i=1}^{n} \hat{z}_{ik}^{(t)}}{n},\ \text{for}\ \ k = 1, \ldots, G.
$$

$$
\begin{aligned}
\frac{\partial}{\partial \boldsymbol{\mu}} Q(\boldsymbol{\mu}|\boldsymbol{x}, \Theta^{(t-1)}) &= \Big(\sum_{i=1}^{n} \hat{z}_{ik}^{(t)} \nu_k (x_i - \mu_k)\Big)_{k=1,\ldots,G} \\
&= \Big(D(\boldsymbol{\nu}^{(t-1)}) Z_t^T \boldsymbol{x} - D(\boldsymbol{\nu}_{Zt})\mu\Big)
\end{aligned}
$$

Since $\boldsymbol{\mu} = M\boldsymbol{\beta}$, by the chain rule

$$
\frac{\partial}{\partial \boldsymbol{\beta}} Q(\boldsymbol{\mu}|\boldsymbol{x}, \Theta^{(t-1)}) = \frac{\partial \boldsymbol{\mu}}{\partial \boldsymbol{\beta}} \frac{\partial}{\partial \boldsymbol{\mu}} Q(\boldsymbol{\mu}|\boldsymbol{x}, \Theta^{(t-1)}) = M^T\Big(D(\boldsymbol{\nu}^{(t-1)}) Z_t^T \boldsymbol{x} - D(\boldsymbol{\nu}_{Zt})\mu\Big)
$$

$$
\frac{\partial}{\partial \boldsymbol{\beta}} Q(\boldsymbol{\mu}|\boldsymbol{x}, \Theta^{(t-1)})_{\boldsymbol{\beta}=\boldsymbol{\beta}^+} = 0 \implies \boldsymbol{\beta}^+ = \Big(M^T D(\boldsymbol{\nu}_{Zt}) M\Big)^{-1} M^T D(\boldsymbol{\nu}^{(t-1)}) Z_t^T.
$$

$$\frac{\partial}{\partial \boldsymbol{\nu}} Q(\boldsymbol{\nu}|\boldsymbol{x}, \tilde{Z}_t, \boldsymbol{\mu}^{(t)}) = \left(\sum_{i=1}^{n} \tilde{z}_{ik}^{(t)} \left(\frac{1}{2\nu_k} - \frac{(x_i - \mu_k^{(t)})^2}{2} \right) \right)_{k=1,\ldots,G}$$

$$\text{where } \tilde{Z}_t = \left(\tilde{z}_{ik}^{(t)} \right)_{1 \le i \le n;\ 1 \le k \le G} \in \mathrm{R}^{n \times G}.$$

$$\frac{\partial}{\partial \boldsymbol{\nu}} Q(\boldsymbol{\nu}|\boldsymbol{x}, \tilde{Z}_t, \boldsymbol{\mu}^{(t)})_{\boldsymbol{\nu}=\boldsymbol{\nu}^+} = 0 \implies \nu_k^+ = \begin{cases} \frac{\sum_i^n \tilde{z}_{ik}^{(t)}}{\sum_i^n \tilde{z}_{ik}^{(t)} (x_i - \mu_k^{(t)})^2}, & \text{hypothesis 1 \& 3} \\ \frac{\sum_i^n \sum_{j=1}^G \tilde{z}_{ij}^{(t)}}{\sum_i^n \sum_{j=1}^G \tilde{z}_{ij}^{(t)} (x_i - \mu_j^{(t)})^2}, & \text{hypothesis 2 \& 4} \end{cases}, \text{ for } k = 1, \ldots, G.$$

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