# The Thesis of the PhD dissertation

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# DEMOGRAPHIC ANALYSIS OF A BUTTERFLY POPULATION

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# 1. BACKGROUND OF THE WORK AND ITS AIMS

In recent decades, the combined effects of global climate change and intensified human land use have led to the loss and deterioration of natural habitats worldwide. To stop biodiversity decline, the most pressing questions today are if and how organisms are able to adapt to these rapid environmental changes and how population viability and individual fitness are affected by these changes.

Butterflies are ideal study organisms to answer both questions, due to their rapid response to environmental variations and ecologically important roles as pollinators and herbivores.

Life history traits, including the number of offspring, age and size at maturity, age-specific mortality (senescence), and investment in reproduction or adult lifespan, impact an organism's ability to survive, reproduce, and, ultimately, contribute to the overall measure of its evolutionary success, i.e. fitness. Reproductive success and survival are considered as major fitness components and they can be estimated by measuring life history traits.

Senescence (or ageing), the deterioration of biological functions with age, a crucial mechanism of decreasing fertility and survival probability through an individual's life, has been a subject of interest in understanding the life history of insects. We distinguish three types of senescence: actuarial senescence (decreasing survival rate), reproductive senescence (reduction of reproductive success) and phenotypic senescence (phenotypic recession).

Reproductive success (and reproductive senescence) is difficult to measure in butterflies (and in insects) in natural populations, because of their small body size, small egg size, high mobility, or cryptic life cycle. In contrast, assessing actuarial senescence by studying apparent survival with mark–recapture method in natural butterfly populations can be easier to implement. Phenotypic senescence has been observed in natural populations of a wide range of vertebrates. However, our knowledge is scarce about it in natural populations of invertebrates. To detect phenotypic senescence, repeated measurements of different body variables along individuals' lifespan is necessary. To date phenotypic senescence has been revealed in laboratory populations of butterflies, and it seems that a relationship between age, body and thorax mass exists, therefore these body sizes may be promising variables for detecting senescence. Wing length and wingspan can also indicate individual fitness.

Environmental factors can influence the fitness of individuals and, together with genetic differences between laboratory and wild populations, can affect ageing rates, so long-term studies in natural populations are needed to correctly assess these life history traits in insects.

## Objectives

My research focuses on the demography analysis of a natural population of Clouded Apollo butterflies (*Parnassius mnemosyne*, Linnaeus 1758, Lepidoptera: Papilionidae), which was studied using mark-recapture and *in situ* non-invasive body size measurements in the field. During my PhD work, I have been looking for answers to the following questions:

- Did body mass and thorax width decline with age (phenotypic senescence)? If so, was this decline linear or polynomial?
- Were there differences between the sexes in initial body sizes and how did body sizes change with age?
- Was the date of first capture of an individual related to its body size and the rate of senescence?
- Was wing length correlated with other body size variables and phenotypic senescence?
- How did survival and recapture probability change with age and the progress of the flight period?
- How were survival and recapture probability related to daily mean temperature and relative humidity?
- How were survival and recapture probability related to initial body mass, initial thorax width, wing length and proboscis length of individual butterflies?

# 2. MATERIALS AND METHODS

## 2.1 Data collection

The Clouded Apollo butterfly is widespread in the Western Palearctic realm. In Central Europe, it inhabits woodland clearings and meadows, rich in flowering plants and open sunny areas, surrounded by woods. This univoltine species is robust enough for repeated measurements of body size without apparent harm, and it is relatively easy to observe, capture and mark them.

Field work was conducted in seven consecutive years between 2014 and 2020 at Hegyesd, a 0.5-hectare meadow in the Visegrádi-hegység, Central Hungary. Insect-pollinated flowering plants were heterogeneously distributed all over the meadow, surrounded by oak wood (*Quercus cerris*).

We sampled the population using mark-recapture (MRR). Sampling was conducted every day during the flight period as weather permitted. We also performed *in situ* non-invasive body size measurements of all newly marked individuals in the field every day, and repeated measurements of body mass and thorax width every third day.

The temperature and relative humidity were recorded with data loggers, installed permanently in the study site.

## 2.2 Statistical analyses

### *2.2.1 Body size change analyses with age*

We defined ‘age’ as the number of days elapsed from the first capture of an individual, which was a minimum estimate for the real age. Age$^2$ was used to model a non-linear relationship between age and body size. ‘Mean(age)’ and ‘mean(age$^2$)’ were the averages of all age and age$^2$, respectively, data at the time of measurements of an individual. These ‘mean variables’ enabled us to distinguish the within-subject and between-subject effects, as mean(age) and mean(age$^2$) are the difference of within-individual effect, and between-individual effect of age (linear) and age$^2$ (quadratic), respectively. The ‘first capture’ variable was the day of the flight period when an individual was captured for the first time, whereas ‘mean(first capture)’ was the annual average of the days of first captures, with which we could test if body size variation among years was related to the variation in flight period length.

Data from the seven study years were pooled and analysed within one model. First, we built a linear mixed-effects model for each response variable (body mass and thorax width) including age, age$^2$, mean(age), mean(age$^2$), first capture, mean(first capture) and wing length, the interactions between these variables and sex, and the interactions between wing length and age and between first capture and age.

We also compared the initial mean values for body mass and thorax width between individuals with only one or repeated (at least two) measurements. Individuals with at least two measurements of body mass and thorax width were only included in this model.

### *2.2.2 Survival analyses*

We used the Cormack-Jolly-Seber (CJS) model for analysing mark-recapture data, excluding the dataset of 2020, because we had no appropriate weather data for this year, therefore we analysed only 6 years of the data (2014–2019). The model has two parameters, apparent survival (‘$\Phi$’ or ‘*Phi*’) and recapture probability (‘$p$’). We used the initial measurements of body mass and thorax width, along with the only measurements of wing and proboscis length as individual covariates in the models. Their quadratic term was also used to model a non-linear relationship between them and the model parameters. We tested the interactions between wing and proboscis and Age as well. For these models, individuals with at least one measurement of all body sizes (initial body mass, initial thorax width, wing and proboscis length) were only included.

‘Day of flight period’ (DOF) was the day in each year when the MRR study was conducted. ‘Age’ was defined as the number of days elapsed from the first capture of each butterfly. Those individuals that were captured, marked and released at the same sampling day, formed a cohort. ‘DOF’, ‘Age’, and ‘Cohort’ were numeric, continuous and time-varying covariates together with weather variables (temperature and relative humidity). They specify whether the given parameter (apparent survival or recapture probability) depends linearly on the days of the flight period, cohort, age or weather parameters. Number of observers was used as a factorial time-varying covariate with three levels: (1) only one observer, (2) two or three observers, (3) four or more observers per day participated in the MRR study. We also used ‘time’ as a factor variable that varies among sampling occasions and it specifies whether apparent survival or recapture probability changed with time.

We aimed to test the relationships between time-varying, and individual covariates, on apparent survival and recapture probability of the butterflies. We used a modified version of the build-up method described in Morin et al., (2020) as a model selection strategy to find those covariates that had a significant relationship with apparent survival and recapture probability. A separate model selection was performed for each year, because generations are not overlapping, and for each sex because of protandry. The model fit was checked by 3 different methods (GOF tests).

# 3. RESULTS AND DISCUSSION

## 3.1 Changes and variability in body sizes

We found a significant decline in body mass and thorax width with age in both sexes, with a non-linear relationship showing slower decline at older ages. The most supported model differed for body mass and thorax width (Table 1); however, random slope models were more supported than random intercept models, indicating individual variance in the rate of body size decline with age.

Body size declined with age slower for males than for females. Individuals captured later in the season had lower body mass and thorax width, and their body mass declined slower with age. Both initial body mass and thorax width were higher with increasing wing length, but wing length had no effect on body size decline.

Table 1: The full and the most supported models' specifications.

| | **Response variable** | **Explanatory variables** | **Random term** |
|---|---|---|---|
| **Linear Mixed-Effects Models (full model)** | **Log(Body mass)** | sex*(age + mean age + age$^2$ + mean_age$^2$ + first capture + mean first capture + wing) + (age:wing) + (age:first capture) | random=~1\|year/id *or* random=~age\|year/id |
| | **Thorax width** | sex*(age + mean age + age$^2$ + mean_age$^2$ + first capture + mean first capture + wing) + (age:wing) + (age:first capture) | random=~1\|year/id *or* random=~age\|year/id |
| **Most supported models** | **Log(Body mass)** | sex*age$^2$ + wing + age*first capture | random=~age\|year/id |
| | **Thorax width** | sex*age + mean age + age$^2$ + first capture + wing | random=~age\|year/id |

Variation and change in butterflies' body mass can be influenced by many factors, i.e. water loss during desiccation, decline in lipid reserves with ageing, oviposition or spermatophore transmission. Clouded Apollo females may receive sphragis (mating plug) during mating, making up to 3–5% of the measured female body mass. This presumably causes body mass loss in males and it may also be a burden for females as flying with sphragis could be costly.

Resource allocation, reserves used from abdominal and thoracic muscles to reproduction or survival may also cause decline, especially in thorax width, however, using thoracic muscles as a resource may be restricted by flight performance.

In addition, high individual variation in initial body size and minor variation in change with age were observed, suggesting a non-negligible individual variance in the rate of body size decline. Sexual dimorphism was also detected, with females having larger initial body mass and thorax width, and a faster decline in body size than males. The presence of such differences may be influenced by many circumstances and can be derived from the dissimilar physiology and behaviour of the sexes.

Newly appearing individuals showed a declining trend in body size with the progress of the flight period, possibly due to a “growth race” among larvae and environmental constraints on development. Later-hatching individuals may face disadvantages, including lower nutrient content in host plants and prolonged larval development.

### 3.2 Survival analyses

The most supported models (Table 2) were not the same for all years, but the directions of the covariates’ effects were similar. Apparent survival declined with age (actuarial senescence) or with the days of the flight period in every year and for both sexes. In most years, survival was negatively related to temperature and positively to relative humidity. And finally, in some years body size was significantly positively related to survival, which means that larger individuals had a higher survival probability.

In most of the years, the most supported models for recapture probability were ‘time’ models, so recapture probability varied among sampling occasions within these years and the covariates could not explain its variance sufficiently. In some years, recapture probability was significantly higher when more observers worked in the field, when temperature was higher and relative humidity was lower. In some other years, recapture probability increased with age and body sizes, i.e. older and/or larger individuals were recaptured with higher probabilities.

Not only those covariates that were included in the most supported models were significant. Likelihood ratio tests resulted in several variables being significant in certain years but not included in the most supported model. This is weaker evidence for an effect than the variables included in the most supported model.

Table 2. Overview of the most supported models of the Cormack-Jolly-Seber model selection. These models were selected based on their AICc values and likelihood ratio tests. (–) negative or (+) positive effects of the variables on apparent survival (Phi) or on recapture probability (p). DOF: Days of the flight period, mass: initial body mass, thorax: initial thorax width, wing: wing length.

| Year | Sex | Apparent survival (Phi) | Recapture probability (p) |
|---|---|---|---|
| **2014** | **males** | ~Age (–) + temperature (–) | ~time |
| | **females** | ~DOF (–) + relative humidity (+) | ~relative humidity (–) + thorax (+) + thorax$^2$ (–) |
| **2015** | **males** | ~DOF (–) + thorax (+) | ~observers (+) + Cohort (–) + wing (+) + wing$^2$ (–) |
| | **females** | ~DOF (–) | ~observers (+) + temperature (+) + thorax (+) |
| **2016** | **males** | ~DOF (–) + temperature (–) | ~time |
| | **females** | ~Age (–) + temperature (–) | ~Age (+) + relative humidity (–) |
| **2017** | **males** | ~DOF (–) + relative humidity (+) | ~time |
| | **females** | ~DOF (–) + relative humidity (+) | ~time |
| **2018** | **males** | ~DOF (–) | ~Cohort (–) + relative humidity (–) |
| | **females** | ~DOF (–) | ~time |
| **2019** | **males** | ~Age (–) + temperature (–) + wing (+) | ~time |
| | **females** | ~Age (–) + temperature (–) + mass (+) | ~temperature (+) |

Circumstances (like weather and available resources, e.g. nectar plants) experienced by individuals were changing from year to year in this natural population, so it could be that the effects of the variables differed between years.

Declining survival probability with increasing age might be due to declining reserves of butterflies which they can use for survival (somatic maintenance). We also revealed phenotypic senescence in this natural population (see the previous section) meaning that these butterflies body sizes declined with age. In the CJS models, we detected lower survival probability with smaller initial body mass and initial thorax width which might mean less reserves. Resource availability during larval and adult stages significantly influences survival and fitness in Lepidoptera. Inadequate resources during these stages often lead to decreased survival rates and compromised fitness in smaller adults.

Being ectotherms means that high daily temperature can cause unfavourable physiological effects in insects including membrane and protein structure disruption, alterations in the cellular microenvironment, DNA damage or elevated metabolic rates, accelerating their ageing process, potentially leading to a decline in survival. At lower relative humidity, in drier conditions, the temperature is higher, therefore water loss increases due to intensified evaporation which may have detrimental effects on butterflies, potentially leading to desiccation stress and increased mortality. Hot and dry conditions were typical at the end of the flight periods, probably that is why we found that survival probability decreased with the progress of the flight period.

Detectability of individuals can be affected by several factors. Presumably, older butterflies are easier to observe due to reduced activity levels, for example, when they are basking with their wings open on the vegetation (dorsal basking). Physiological deterioration, such as thorax muscle atrophy and declining energy reserves likely contribute to this decreased activity, resulting in reduced energetically demanding behaviours such as flight or foraging in older individuals. Alternatively, older females may fly weaker and thus more likely to be captured. Conversely, observers might also detect more active individuals that fly frequently and are more visible with a higher probability.

We note that body size parameters did not appear in the most supported models in every study year and their effects on survival and recapture probability were rather weak in some cases. The interaction between age and wing length, and age and proboscis length was not significant in any year.

## 4. CONCLUSION AND RECOMMENDATIONS

We conducted a long-term (7 consecutive years) study in a natural population of Clouded Apollo butterflies using mark-recapture method complemented with continuous recording of weather parameters and precise measurements of body size of individual butterflies.

To our knowledge, this is the first study that revealed phenotypic senescence in a natural butterfly population, using *in situ* non-invasive measurements. Furthermore, we were able to detect actuarial senescence and revealed how body size and weather variables affected Clouded Apollo butterflies' survival and recapture probability.

Our results suggest that the rates of senescence and larval growth may be influenced by individually and annually varying environmental variables. Body size largely determines fecundity and senescence may also affect reproductive success in insects. Therefore, we recommend that laboratory studies in the future should aim to (i) uncover the separate effects of certain weather conditions (temperature, humidity etc.) and resource availability on the rates of larval growth, adult survival and senescence, (ii) investigate the genetic background and its relationship with environmental factors of ageing of insects. In addition, more long-term field studies in natural populations of butterflies should be conducted in order to reveal the combined effects of different environmental factors on the survival probability of individuals. Ultimately, a deeper understanding of these relationships would help us better predict the effects of current global environmental changes on the viability of insect populations.

If we knew the vulnerabilities of these butterflies, it would be easier to take the appropriate steps to mitigate the effects of climate change and habitat loss, e.g. with appropriate habitat management.

For Clouded Apollos, the most important thing is the patchiness of the habitat heterogeneity with open, sunny spots and with herbaceous plants interspersed with shrubs, as well as the transition zone between the meadow and the forest, where they lay their eggs near to *Corydalis* species.

Understanding the interplay between survival, life history traits, and fitness is essential for comprehending the adaptive strategies of Lepidoptera in response to environmental pressures and for predicting their population dynamics in changing ecosystems. In order to detect trends and control for intra- and interspecific longevity and ageing, longitudinal research in consecutive generations is necessary. These scientific results can later be applied to conservation biology research and management plans for species conservation programmes. Future research could play a major role in determining the maximum temperature that the species can tolerate, which, combined with local climate predictions, could help to select habitats where the studied butterfly population can be protected and sustained in the long term.

## 5. NEW SCIENTIFIC RESULTS

1) This is the first study where body mass and thorax width were repeatedly measured on butterflies during their lifespan *in situ* non-invasive in their natural habitat.
2) We revealed phenotypic senescence in a natural population of Clouded Apollo butterflies. We detected that both body mass and thorax width decreased with age, proving that physiological ageing exists in natural circumstances in butterflies. We found sexual differences in the rate of phenotypic senescence, but despite the annual variation of initial body sizes, the rate of senescence did not vary considerably across years.
3) We provided evidence that actuarial senescence occurred almost every year (2014–2017 and 2019) in this natural population of Clouded Apollos, which means that the survival probability of butterflies decreased with age.

4) We found that ambient temperature and relative humidity significantly influenced butterfly survival. Butterflies had higher survival at cooler temperature and higher relative humidity.

5) We also found that body size, especially thorax width and wing length, was positively related to butterfly survival in some years, indicating that larger butterflies survived better.

6) The effects of weather variables and body size were more apparent at an older age of Clouded Apollos.

7) In some years, detectability of butterflies was significantly affected by weather parameters and body size. The recapture probability was higher at higher temperatures and lower relative humidity, as well as in larger butterflies.

# 6. THE PUBLICATIONS OF THE AUTHOR IN THE RESEARCH FIELD

## Peer-reviewed papers published in international scientific journals:

Phenotypic senescence in a natural insect population
**Pásztor K**, Kőrösi A, Gór A, Szigeti V, Vajna F, Kis J; Ecology and Evolution12 e9668, 2022 (IF$_{2022}$: 2.600)

Mate-guarding success depends on male investment in a butterfly
Gór A, Lang Zs, **Pásztor K**, Szigeti V, Vajna F, Kis J; Ecology and Evolution 13 e10533, 2023 (IF$_{2022-23}$: 3.167)

Facultative male investment in prolonged mate-guarding in a butterfly
Gór A, Fónagy A, **Pásztor K**, Szigeti V, Lang Zs, Kis J; Behaviour 160(6) 515-557, 2023 (IF$_{2022-23}$: 1.672)

## Peer-reviewed papers published in Hungarian scientific journals:

Változó hím befektetés a nőstény további párosodását akadályozó képletekbe kis apollólepkénél (Parnassius mnemosyne (LINNAEUS, 1758))
Gór Á, Fónagy A, **Pásztor K**, Szigeti V, Lang Z, Kis J; Állattani Közlemények 108 (1-2), 2023

## Proceedings in international conferences

How weather and body size affect survival and detectability in a natural butterfly population – **Pásztor K**, Kőrösi A, Gór A, Kis J –8th Student Conference on Conservation Science Europe - Balatonvilágos, Hungary, 2023 – Talk (Best Talk Award)

How weather and body size affect survival, senescence and detectability in a natural butterfly population? – **Pásztor K**, Kőrösi A, Gór A, Kis J – International Conferences on the Biology of Butterflies - Prague, Czech Republic, 2023 - Poster

Change of body mass and thorax width with age in a natural butterfly population – **Pásztor K**, Kőrösi A, Gór A, Kis J – 7th Student Conference on Conservation Science Europe - Balatonvilágos, Hungary, 2022 – Poster (Best Poster Award)

Multilevel variation in body mass and thorax width in a natural butterfly population – **Pásztor K**, Kőrösi A, Gór A, Kis J – XXII European Congress of Lepidopterology - Laulasmaa, Estonia, 2022 - Talk

Change of body mass and thorax width with age in a natural butterfly population –**Pásztor K**, Kőrösi A, Gór A, Kis J – Annual Meeting of the Lepidopterists' Society (Virtual), 2021 – Poster (Awarded Poster Presentation, 2nd place)