University of Veterinary Medicine Budapest
Doctoral School of Veterinary Science

THE REPRODUCTIVE SYSTEM OF CLOUDED APOLLOS (*PARNASSIUS MNEMOSYNE*)

PhD dissertation

Ádám Kristóf Gór

2025

Supervisors

……………………………….	……………………………….
János Kis, PhD	Zsolt Lang, PhD
Department of Zoology	Department of Biostatistics

Copy … of four.

………………………………………….

Ádám Kristóf Gór

Table of content

Table of figures

List of abbreviations and terms

1. **CAP**: Copulatory opening APpendix, the varieties of the externally visible male-produced devices that may impede the female Clouded Apollo butterflies' future mating.

2. **No CAP**: no Copulatory opening APpendix can be observed externally in the copulatory opening.
3. **Filament**: a thin, elongated, thread-like Copulatory opening APpendix, extending from the copulatory opening; the sinus of the opening is not filled.
4. **Stopple**: a compact Copulatory opening APpendix that at least partially fills the sinus of the copulatory opening. It may extend well over the copulatory opening and cover the sinus entirely.
5. **Small CAP**: a term consisting both filaments and stopples.
6. **Shield**: a sheet warped around and attached to the stopple with lateral bridges. It is much larger than the stopple and may cover most of the ventral abdomen.
7. **Sphragis**: the complex of the stopple and shield. It is the generally used term in the literature for mate-guarding devices found in sphragis-bearing butterflies.
8. **CCF**: cross-correlation function is a measure of similarity of two series as a function of the displacement of one relative to the other.
9. **ASR**: adult sex ratio (all alive males / all alive males + all alive females). Daily ASR is the ASR calculated for a given day.
10. **Shield-to-CAP ratio**: the number of shields observed per day divided by the total number of daily Copulatory opening APpendices. Daily shield-to-CAP ratio is the shield-to-CAP ratio calculated for a given day.

1. Summary

Sexual conflict refers to the evolutionary struggle between males and females over reproductive resources and strategies. One manifestation of this conflict is mate-guarding, a behaviour observed in many species where males actively prevent other males from accessing a female to ensure their paternity. This protective behaviour is driven by the male's motivation to maximise its reproductive success and minimise the risk of females mating multiple times. In some species, mating plugs add another layer to the sexual conflict dynamics. Mating plugs are structures or substances deposited by males in the female reproductive tract to impede the access of rival males, reducing the likelihood of sperm competition. This adaptation serves as a form of post-copulatory mate guarding, ensuring that the male's genetic material has a better chance of successfully fertilising the eggs.

Males of many insects, including butterflies, produce mate-guarding devices, such as mating plugs, to prolong guarding and prevent future female matings in the male's absence. In most Lepidoptera, internal plugs are common, while in two butterfly families, large external devices, called sphragides, evolved independently. The lack of, or incomplete sphragis in a few mated females were reported in sphragis-bearing species.

Previous studies focused on typifying species-specific mate-guarding devices in a few specimens of many species. In contrast, we investigated a sphragis-bearing butterfly’s alternative mate-guarding devices in detail and behaviours related to those, and aimed to introduce their English and Hungarian terminology. We conducted an extensive observational field study through six years based on mark-recapture in a natural Clouded Apollo (*Parnassius mnemosyne*) population. Inspecting 492 females, we identified three different types of devices, termed as Copulatory opening APpendices (CAP). Filaments, stopples (together named as small CAPs) and shields (i.e., sphragides) increased in size and structural complexity in the respective order, implying differential male investment and effectiveness in securing paternity. Shield dimensions, colour and all devices’ shapes varied considerably. Some devices were lost, and a few were video-recorded when removed by males, showing the role of different parts of the male external genitalia.

Our further aim was to reveal CAP-replacement dynamics within females during their lifetime and to understand how male investment into small CAPs or shields was (i) related to CAP persistence on the female, i.e., securing paternity, (ii) associated with female quality, measured as size and (iii) with actual adult sex ratio. To estimate CAP-replacement risks, we used multistate survival models. Shields were the most frequent mate-guarding devices and were more persistent than small CAPs, often lasting for life, excluding future matings. The ratio of shields to all CAPs gradually decreased towards the end of the flight period. Females were more prone to receive a

shield when they had wider thoraces, compared to the narrow, and when the ratio of males (i.e. male-male competition) was higher in the population.

Direct observations and video recordings indicate different and multiple roles of the valvae (the male copulatory organ) and the aedeagus (another part of the male external genitalia) in producing, fastening and removing CAPs. We predict that alternative mate-guarding devices similar to that of *P. mnemosyne* could be found under scrutiny in more species known for sphragis bearing. We assume that the final outcome of CAP production, i.e. the CAP-type produced and the size of the CAP depends on the relative quality of the mates at an actual mating attempt, as well as operational sex ratios and the progress of the flight period. We propose that at high male-male competition, if a male can afford, produces a shield, but if medium competition occurs, produces a shield for a large female or a small CAP for a small female and if competition is low, produces a small CAP. Since larger females were more prone to receive a shield than a small CAP and shields mostly lasted for life while small CAPs were often replaced, we further assume that ‘high quality’ females were more deprived of postcopulatory female choice and genetic variance in their offspring may be reduced compared to the ‘low quality’, therefore more polyandrous females.

To our best knowledge, this is the first quantitative study to investigate potential factors on which male butterfly investment in mate-guarding devices may depend, and how the variation in these devices impacts CAP-persistence on females.

2. Introduction

Anisogamy, the condition where gametes (reproductive cells) of the two sexes differ in size, is a fundamental aspect of reproductive biology in animals. This phenomenon contrasts with isogamy, where gametes are of equal size (Togashi et al. 2012). In most anisogamous species, eggs (i.e. ova), the much larger and often immobile gametes, require large amounts of material and energy to produce. In contrast, sperm cells, the smaller and more mobile gametes, are numerous and require less investment for production (Togashi et al. 2015). This fundamental difference in gamete size sets the stage for diverse reproductive strategies (Parker et al. 1972, Seed & Tomkins 2018). The evolution of anisogamy has profound implications for reproductive strategies, influencing mate selection, parental investment, and overall reproductive success (Charlesworth & Charlesworth 2010). The research by Parker et al. (1972) lays a fundamental groundwork for understanding the evolutionary basis of anisogamy. Parker's theory emphasises the differential investment between sexes in reproduction. Anisogamy is prevalent across the animal kingdom, from simple multicellular organisms to complex vertebrates (Bell 1978).

Sexual conflict in the animal kingdom originates from anisogamy and arises from the inherent differences in reproductive strategies between males and females (Arnqvist & Rowe

2002, Martin & Hosken 2003, Parker 1979, 2006, Partridge & Hurst 1998, Tregenza et al. 2006), leading to a dynamic interplay shaped by evolutionary pressures. Bateman's principle, proposed by A. J. Bateman in 1948, lays the groundwork for understanding the basis of sexual conflict. This principle suggests that females, with their higher investment in producing a gamete, become the limiting resource for males, fostering competition among males for access to mates (Bateman 1948).

Male-male competition is a common manifestation of sexual conflict, driving the evolution of exaggerated secondary sexual characteristics. These traits, such as elaborate courtship displays and vibrant plumage, are often showcased by males to attract females. The work of Andersson (1994) provides comprehensive insights into the evolution of such secondary sexual characteristics, emphasising their role as signals of genetic quality or the ability to provide resources. These traits not only facilitate mate attraction but also highlight the competitive nature of male-male interactions. In species where direct physical combat determines access to mates, weapons for combat may evolve. This can include structures like antlers in deer or horns in beetles, which are used as tools for intra-specific combat. The evolution of these structures is shaped by the need to establish dominance over rivals and secure mating opportunities (Emlen 2008).

On the female side, resistance mechanisms have evolved as a countermeasure to male reproductive strategies. Female resistance can take various forms, including physical struggles and cryptic female choice. This dynamic is detailed in the work of Arnqvist & Rowe (2005), which delves into the complexities of sexual conflict, exploring how females actively shape their reproductive outcomes. Mate choice is a pivotal aspect of sexual conflict. It refers to the selection of mates based on certain traits or behaviours, and it is often a source of conflict between males and females (Andersson & Simmons 2006). In many species, females are selective in choosing mates, seeking traits that indicate genetic quality or the ability to provide resources. This selectivity can lead to the evolution of exaggerated secondary sexual characteristics in males (Andersson 1994). These traits serve as signals that females use to assess the fitness of potential mates.

Cryptic female choice represents a fascinating and intricate aspect of reproductive dynamics in the animal kingdom, highlighting the active role females play in shaping their reproductive outcomes (Firman et al. 2017). This phenomenon refers to the post-copulatory processes through which females influence paternity or manipulate the utilisation of male sperm, often occurring in a concealed manner. A work in understanding cryptic female choice is presented by Thornhill & Alcock (1983). The authors discuss how, beyond the point of copulation, females possess mechanisms to exert selectivity in fertilisation. This selectivity can involve sperm choice, where females bias the usage of sperm from certain males over others, influencing the genetic makeup of the offspring.

One mechanism through which cryptic female choice operates relies on the sperm storage organs. In many species, females possess specialised storage organs where sperm can be stored for extended periods. Birkhead & Pizzari (2002) delve into the intricacies of these structures and how females may selectively use or discard stored sperm based on post-copulatory cues of female quality. This storage process provides females with the opportunity to assess the quality of the sperm and potentially favour the sperm from certain males over others. Moreover, the female reproductive tract itself can act as a selective environment. In species where multiple males mate with the same female, the competition between sperm from different males occurs within the female reproductive tract. The work of Eberhard (1996) emphasises the significance of female-controlled processes that influence the success of particular sperm, demonstrating the active role females play in determining the genetic composition of the offspring. Cryptic female choice extends beyond sperm selection to include mechanisms that influence fertilisation success. For instance, in certain species, females can manipulate the timing of fertilisation, ensuring that sperm from a preferred mate has a higher likelihood of fertilising the eggs (W. G. Eberhard 1996).

Cryptic female choice challenges traditional views of reproduction by highlighting the complexity of post-copulatory interactions. It underscores that the female's reproductive tract is not merely a passive conduit for sperm, but an active arena where females can exert influence over the genetic outcome of mating. Thornhill & Alcock's (1983) pioneering work, along with contributions from (Birkhead & Pizzari 2002) and (W. G. Eberhard 1996), forms the foundation for our understanding of cryptic female choice, shedding light on the strategies females employ to maximise their reproductive success.

The dynamics of sexual conflict often lead to a coevolutionary arms race, a phenomenon where one sex evolves traits or behaviours to counter the adaptations of the other (Parker 1983). The concept of an evolutionary arms race describes the perpetual antagonism between species, particularly predators and prey, or in the context of sexual selection, between the sexes of the same species, resulting in an ongoing cycle of adaptation and counter-adaptation (Tewksbury 2002). This dynamic process is driven by the competition for survival and reproduction, leading to the development of increasingly sophisticated traits and strategies (Areja-Gavina et al. 2021, Janicke & Fromonteil 2021).

In the realm of sexual selection, an evolutionary arms race unfolds between males and females, particularly evident in traits and behaviours associated with mate choice and reproductive success. This interplay is well illustrated by the work of Gavrilets et al. (2001), who discuss how certain species engage in a coevolutionary arms race in the context of sexual conflict. Males may develop strategies and traits to enhance their reproductive success, prompting females to evolve counter-strategies, leading to a continuous cycle of adaptations and counter-adaptations. A classic example of an evolutionary arms race is observed in the development of genitalia. The work of Eberhard (1985) highlights how genital structures in insects, such as the elaborate male genitalia of certain species, evolve in response to female reproductive tract structures. Females, in turn, may evolve mechanisms to counteract or control male genitalia, creating a dynamic cycle of evolutionary change.

Guarding may pay off to secure and maintain exclusive access to a mate, ultimately enhancing reproductive success and it is an outcome of sexual conflict in animals (Benvenuto & Weeks 2012, Parker 1970, Simmons 2002). Male mate-guarding has been found in a broad variety of taxa from rotifers (Schröder 2003) to primates (Alberts et al. 1996) and can be performed before mating (precopulatory mate-guarding; Jormalainen 1998) in order to ensure a mate, or after mating (postcopulatory mate-guarding; Sakaluk 1991) to prevent the mate from remating. Mate guarding strategies can manifest in different forms, ranging from physical aggression to vigilant proximity, and they provide insights into the dynamic interplay between males and females in the context of reproductive competition.

One primary motivation behind mate guarding is the need to minimise the risk of sperm competition, where multiple males compete for fertilisation opportunities within the female reproductive tract (Birkhead & Pizzari 2002). By preventing access to potential rivals, males can increase the likelihood of their sperm successfully fertilising the eggs. Birkhead and Pizzari's (2002) comprehensive exploration of sperm competition highlights the significance of mate guarding as an adaptive response to reproductive challenges. Territorial mate guarding is a common strategy observed in many species. Males establish and defend territories that encompass critical resources or desirable mating sites, restricting the access of competing males. The work of Davies (1985) elucidates the importance of territories in mate guarding, emphasising how the establishment and maintenance of exclusive territories contribute to reproductive success. Vigilant proximity is another form of mate guarding, where males stay in close proximity to females to deter other males from approaching. This behaviour is observed in various taxa, including birds and mammals. By maintaining constant proximity, males can minimise the chances of surreptitious copulations by rival males, ensuring their own reproductive success (Birkhead & Pizzari 2002).

In certain cases, mate guarding involves physical aggression directed towards potential rivals. This aggressive mate guarding behaviour is seen in species where males engage in direct combat to assert dominance and secure mating opportunities. Such aggressive interactions are often observed in polygynous mating systems, where one male mates with multiple females (Clutton-Brock 1989). The study by Clutton-Brock (1989) provides valuable insights into the link between mating systems and the intensity of mate guarding strategies.

Mate guarding is not limited to the male's perspective. Females may also engage in mate guarding behaviours to ensure the fidelity of their chosen mate. In some cases, female mate guarding involves active participation in the exclusion of rival females from the vicinity of their mate (Arnqvist & Rowe 2005).

The investment in guarding may depend on several factors. (i) The level of competition among males for mating increases with the increasing ratio of males in a population (Weir et al. 2011), thus selecting for males to invest more in mate-guarding (Jormalainen 1998, Takeshita & Henmi 2010). In insects, last male sperm precedence is common, when the last mated male fertilises most of a multiply mated female's eggs (Boggs & Watt 1981, Parker 1970, Simmons 2001, Sims 1979, but see Tregenza & Wedell 2002), thus male benefits from postcopulatory mate-guarding are further enhanced (Alcock 1994). (ii) The amount of affordable resources and time to spend on guarding may depend on individual variation (Cueva Del Castillo 2003), e.g. condition among competitors. (iii) The expected reproductive value of the mating partner (Manning 1975, Schöfl & Taborsky 2002, Shuster 1981, Thompson & Manning 1981) may also depend on individual variation, e.g. the variance in the conditions among potential partners. The latter may result in males assessing female quality before mating. In insects, the general pattern is that larger female body size is associated with better quality (Gilbert 1984, Honěk 1993, Oberhauser 1997, Okada et al. 2021, Prenter et al. 1994, Sibley et al. 2001), i.e. higher fecundity, consequently more eggs produced during lifetime. Therefore, males may benefit from investing more in the guarding of larger than smaller females (e.g. Knox & Scott 2006). Besides the guarding males, guarded females may also incur benefits, by avoiding further harassment or even injuries from other males (Dickinson & Rutowski 1989, Kawagoe et al. 2001, Nilakhe 1977, Orr 1999, Thornhill & Alcock 1983). However, mate-guarding prolonged over a considerable period, including the entire adult life of a female, may incur costs by depriving her from multiple matings. These costs may be the reduced amount of resources such as spermatophores from other males (L. D. Marshall & McNeil 1989), reduced opportunities for postcopulatory mate choice (Firman et al. 2017) and reduced genetic benefits of its progeny (Fedorka & Mousseau 2002).

For the guarding male, the time and resources invested in the extension of the mate-guarding period would pay off (King & Fischer 2005) if it could produce a device that blocks the copulatory opening of its mate and guard it while not being there. Mating plugs are postcopulatory devices considered to impede or block the females from future matings, and are inserted into the female copulatory organ (Stockley et al. 2020). These devices are taxonomically widespread, described from nematodes (Timmermeyer et al. 2010) to primates (Danzy et al. 2009, Dixson & Anderson 2002, Dunham & Rudolf 2009). Mating plugs play a crucial role in influencing reproductive outcomes by potentially impeding the access of rival males to a recently mated female and affecting the chances of successful fertilisation (Sutter & Lindholm 2016). These plugs have been shown to reduce the likelihood of subsequent matings by other males and may serve as a mechanism to ensure the paternity of offspring (Sauter et al. 2001).

The formation and persistence of mating plugs raise intriguing questions about their adaptive significance. One prevailing hypothesis is that mating plugs function as a strategy to reduce the risk of sperm competition (Birkhead 1995, Parker 1984). By physically obstructing the female's reproductive tract, males can potentially hinder the movement of rival sperm and increase the likelihood of their own sperm fertilising the eggs. However, the efficacy of mating plugs varies among species, and their presence does not guarantee reproductive success (Keller & Reeve 1995, Matsumoto & Suzuki 1992). Some females may possess mechanisms to remove or bypass mating plugs (Polak et al. 2001), while males may evolve counter-strategies to enhance the effectiveness of their plugs, and at the same time to be able to remove enhanced mating plugs produced by rival males. This dynamic interplay between the sexes underscores the complexity of reproductive strategies in the context of sperm competition.

In most lepidopteran taxa, internal plugs are common (Matsumoto & Suzuki 1995, Orr 1995), while in two butterfly families, Nymphalidae and Papilionidae, large, structured external mate-guarding devices, called sphragides (singular: sphragis), evolved independently of each other (Carvalho et al. 2017). The term ‘sphragis’ originates from the Greek word meaning ‘seal’, and it describes the function of this structure. Sphragides are waxy or proteinaceous substances secreted by males, covering the female copulatory opening and may persist on females throughout their postcopulatory life (Carvalho et al. 2017, Matsumoto & Suzuki 1995, Orr 1995). These devices do not block oviposition since most lepidopterans are ditrysian, i.e. the copulatory opening is separated from the oopore (Scoble 1992). From internal plugs to large elaborate sphragides, various, species-specific forms exist in butterflies, differing in size relative to body size, structure, composition, shape, colour, as well as the means how males produce and fix them on the female (Carvalho et al. 2017). In many insects, including butterflies, as well as sphragis-bearing species, cryptic female choice involves the storage of sperm within specialised structures known as spermathecae. The spermatheca serves as a reservoir for received sperm, allowing females to control the timing and utilisation of sperm for fertilisation (Pérez- Staples et al. 2007, Watanabe 2016). There is evidence that the females of sphragis-bearing butterflies, despite being sealed with sphragides, may mate multiple times (Matsumoto 1987, Vlašánek & Konvička 2009) allowing them to choose between male spermatophores, i.e. capsules or masses containing sperm.

Although the study of sphragides, including arguments on how these prevent butterfly remating, has been started more than a century ago (e.g. Bryk 1918, 1919, Marshall 1901), data on within-species size and morphological variation are scarce (Carvalho et al. 2019). To our best knowledge, quantitative studies on how this variation impacts the guarding devices’ persistence on females are absent to date. Moreover, previous studies on sphragides aimed at typifying and comparing species-specific structures within a range of taxa, and understanding their formation and evolution (Carvalho et al. 2017, 2019, Matsumoto 1987, Matsumoto & Suzuki 1995, Orr 1988, 1995, 2002). Several studies reported the lack of sphragis in a few individuals of sphragis-bearing species considered lost for various reasons (Marshall 1901, Petersen 1928, Matsumoto unpublished data), either attributed to removal (Matsumoto & Suzuki 1992, 1995, Vlašánek & Konvička 2009) or spontaneous loss (Pierre 1985, Vlašánek & Konvička 2009). Most females bear sphragides in the Apollo butterflies (*Parnassius*, Papilionidae, except the subgenus *Sachaia* (Carvalho et al. 2017), but sphragides have not been found in a small proportion of the females in natural populations of *Parnassius mnemosyne*, L. (Vlašánek & Konvička 2009), *Parnassius clodius*, M. (Auckland et al. 2004, Calabrese et al. 2008) and *Parnassius smintheus*, D. (Calabrese et al. 2008, Matter et al. 2012). There is also evidence of ‘incomplete sphragides’, which are conspicuously smaller sphragides than the typical size characterising a species and attributed to material resource depletion in multiply mated males (Carvalho et al. 2017, Matsumoto 1987, Orr 1988, 2002).

We conducted a detailed observational field study in a small Clouded Apollo butterfly (*Parnassius mnemosyne*) population throughout several consecutive years in a semi-natural habitat. In Hungary, the Clouded Apollo butterfly is abundant in most suitable habitats and has many small, closed populations where the majority of females can be captured and scrutinised. The lack of sphragis in some females (Vlašánek & Konvička 2009) together with ‘incomplete sphragides’ reported in other related species (Carvalho et al. 2017, 2019, Matsumoto 1987, Orr 1988, 2002) made the Clouded Apollo a promising candidate to investigate facultative male investment into mate-guarding.

2.1. Main goals

The primary objective was to investigate and understand the Clouded Apollo butterfly’s (*Parnassius mnemosyne* L.) reproductive system and its dynamics by conducting a multi-year study on a natural population. Our aims were to

1. describe the variability and peculiarities of mate-guarding devices,
2. since we found morphologically different devices besides the well-known sphragides, provide their detailed structural descriptions,
3. introduce the terminology of these devices both in English and in Hungarian,
4. discuss mate-guarding devices in relation to male and female anatomy,
5. describe behaviours related to mating, device production and device removal attempts based on direct observations, photographs and video recordings,
6. reveal how male investment into different devices was (i) related to securing paternity, (ii) associated with female quality and (iii) with actual adult sex ratio (ASR).

We expected large variation in CAP size and shape as males have varying reserves that can be allocated to mate-guarding. Furthermore, we assumed that males invest more in larger than smaller females, since larger female insects were found to be more fertile. Additionally, males were expected to invest more in guarding females at higher rates of male-male competition, i.e. male-biased sex ratios. Finally, we predicted that males invest more in mate-guarding early in the flight period than later, since they are younger and have more reserves, and also encounter younger and larger females which are more fertile, thus more rewarding for the males.

3. Material and methods

3.1. Study species

The Clouded Apollo is a Eurasian butterfly depending on open habitats within deciduous forests (Konvička & Kuras 1999, Meier et al. 2005, Weiss 1999). Abundant in many of its habitats, this univoltine species flies from late April to the beginning of June in Hungary (Gergely et al. 2018), thus adult generations do not overlap. The egg is the overwintering stage (Bergström 2005) and larvae feed in the spring on *Corydalis* DC. (Papaveraceae) species. The sexes are easy to distinguish, since the dorsal thorax and the abdomen in males are densely covered with hair, while almost bald in females, and females have yellow scales on the sides of their abdomen and the back of the head, absent in males (**Figure 1**). Adults spend much time feeding on nectar plants (Konvička & Kuras 1999, Szigeti et al. 2018, Vojnits & Ács 2000). Clouded Apollos are protandrous, i.e. males on average emerge earlier than females during the flight period (Bella 2017, Szigeti et al. 2019, Vlašánek & Konvička 2009). Males often patrol seeking females whom they usually force to mate and a sphragis may be formed towards the end of mating (Konvička et al. 2001, Konvička & Kuras 1999). Mated females lay eggs several times during their life (Meglécz et al. 1999).

The Clouded Apollo is threatened across Central Europe by forestry intensification and protected under the Bern Convention (van Swaay et al. 2010), although may be abundant in suitable habitats in Central Europe (Konvička & Kuras 1999, Ronkay 1997, Weiss 1999).

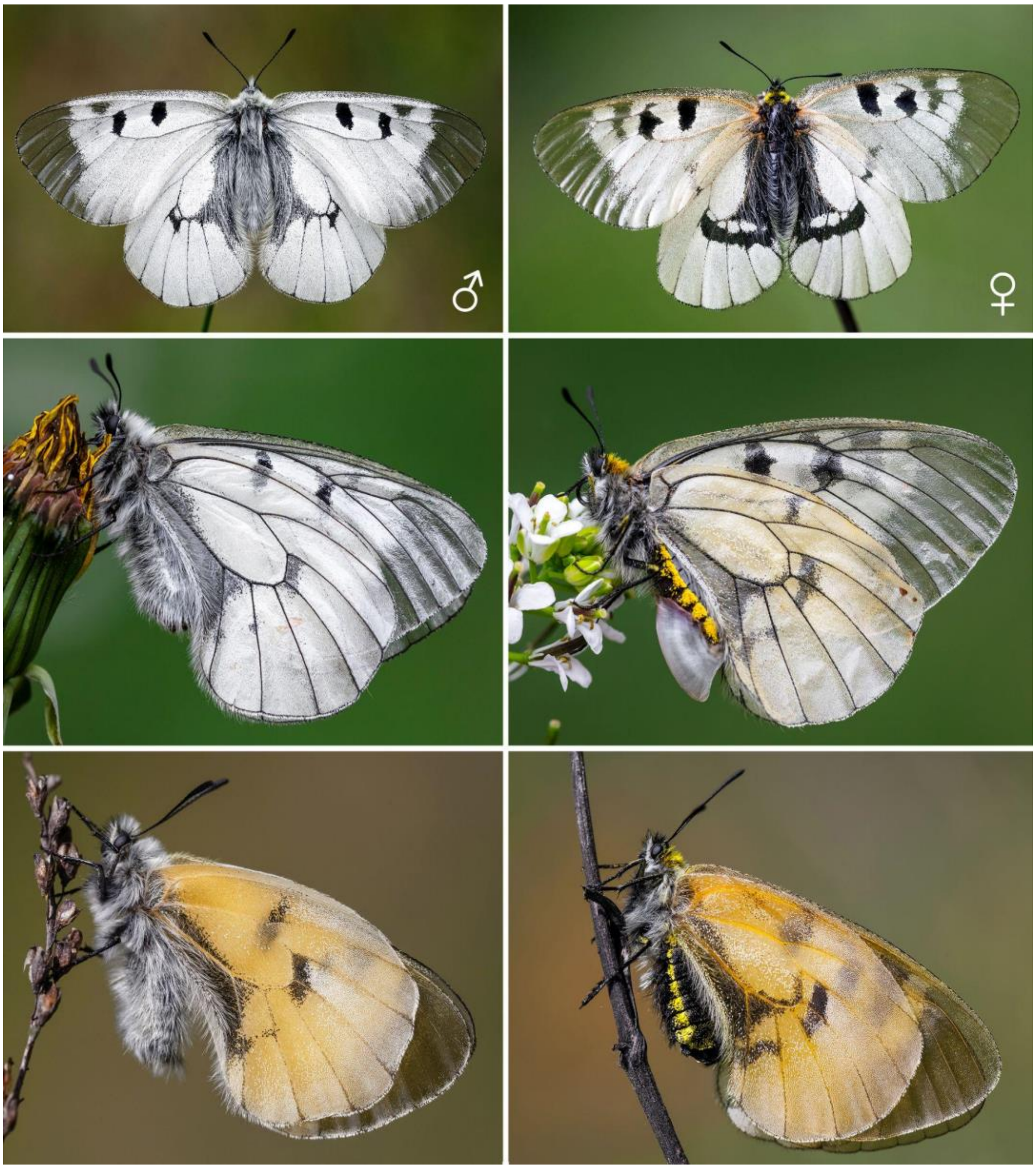


Figure 1. Clouded Apollo males (left) are densely covered with hair, while females (right) are almost bald, and have yellow scales on the sides of the abdomen and the back of the head, absent in males. Females may bear a sphragis on their abdomen. Both sexes have yellow tinted wings soon after emergence (bottom). Photos were taken by ÁG.

3.2. Study site and period

Field work was carried out at Hegyesd, a 0.5-hectare colline meadow, surrounded by a Turkey oak (*Quercus cerris* L.) forest (**Figure 2, Figure 3**) in the Visegrádi-hegység, Hungary, Central Europe (47.756411, 19.047897, at 295 m a. s. l.). Observations began when the first Clouded Apollo adults appeared and lasted until the last individual was on wing between 2015 and 2020 (**Table 1**). We sampled butterflies between 9 AM and 6 PM all days of the Clouded Apollo's flight period, as weather permitted.

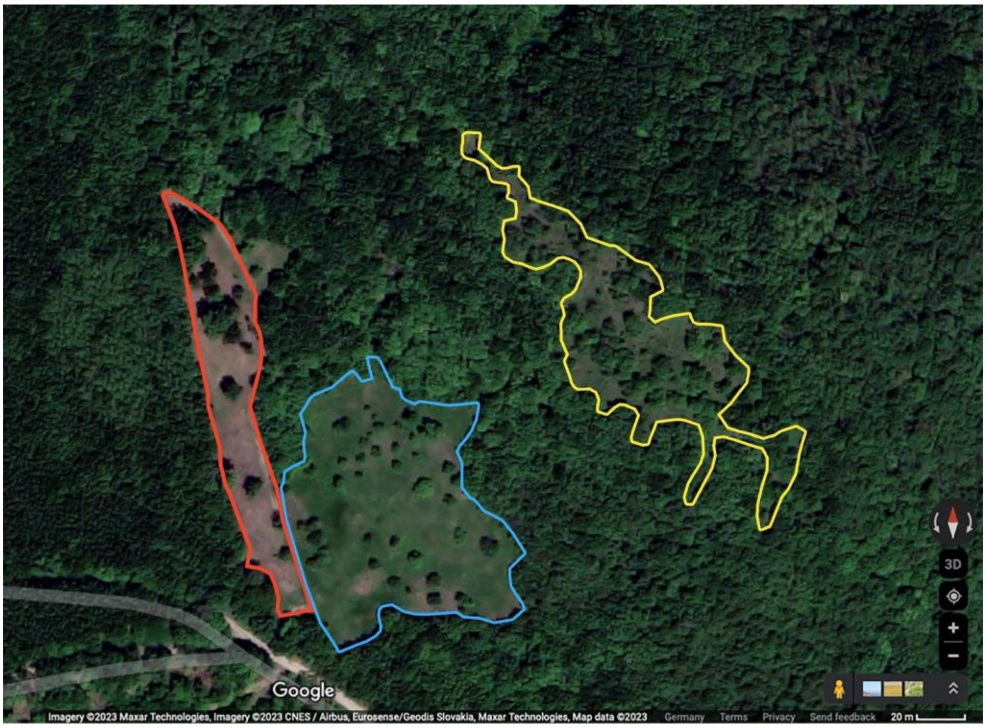


Figure 2. Aerial photograph on the study site (Hegyesd, Visegrádi-hegység) and its surroundings (obtained via © Google Maps, 2023). Yellow denotes the area where most Clouded Apollos were caught and observed, a hilltop and a SW slope. Blue denotes a seemingly suitable habitat at a lower elevation, where Clouded Apollos were only occasionally caught or observed. Red denotes a fresh clearing (2020) without suitable forbs for Clouded Apollos.

Figure 3. Habitat photographs of the study site. Note the patchy character of the open meadow, scattered with small groups of trees and shrubs. Photos were taken by JK.

Table 1. Annual variation in the lengths of the flight periods, the number of individuals, and the number of Clouded Apollo females captured.

Year	First day	Last day	Duration (days)	$n_{individuals}$ (total = 1108)	$n_{females}$ (total = 492)
2015	2015-04-26	2015-05-30	35	176	84
2016	2016-04-22	2016-06-03	43	194	88
2017	2017-04-25	2017-05-29	35	189	87
2018	2018-04-29	2018-05-24	26	271	116
2019	2019-04-21	2019-06-04	45	203	83
2020	2020-04-21	2020-05-21	31	75	34

3.3. Sampling

Mark-release-recapture (MRR) was used to survey the population. We aimed to capture all unmarked butterflies with a butterfly net. We marked them individually with a colour combination on both forewing tips with edding® paint markers, gave an identification number on both hindwings (**Figure 4**), marked new sphragides with black dots (edding® OH permanent marker; both inside (that has never been lost), for better persistence, and outside, for better visibility, of the sphragis wall; **Figure 4**) and then released them (Szigeti et al. 2018). Black dots allowed identifying already observed sphragides. Females with unmarked sphragides that previously had a marked sphragis, lost the sphragis first observed, then received a new one. We monitored the meadow regularly throughout the day, recorded marked females and checked markings on the sphragides. Furthermore, we attempted to capture all marked females with unmarked sphragides, as well as all marked females without sphragides once a day. For the survey, observers followed the same routes which had been systematically distributed in the meadow to reduce trampling (Szigeti et al. 2016).

Figure 4. Our markings on a Clouded Apollo female. Note the identification number on the ventral side of the hindwing, the colour combination on the tip of the forewing and the black dots on the sphragis. Photo was taken by ÁG.

3.4. Brief introduction to the terminology

We found small, presumably mate-guarding devices in most of the females. This is one of our key findings I briefly present here, because it is necessary to understand some parts of the methodology. I present these in full detail in the Results' 4.1.1. Terminology section. We termed the varieties of the externally visible male-produced devices that may impede the female's future mating as a Copulatory opening APpendix (CAP). CAPs in this species consist of three types, the filament, the stopple and the shield. Filaments are small threads found in the female copulatory opening. Stopples are a little larger appendices that, in contrast to filaments, cover the opening entirely. Filaments and stopples are together named small CAPs. The shield is a warped sheet built on top of a stopple, assumed to prevent stopple removal by rival males, and is much larger than small CAPs. The shield approximately corresponds to the term sphragis (sensu Carvalho et al. 2017; see also Orr 1995). Virgin females start their life with no CAP, i.e. no appendix can be observed externally in the copulatory opening. Although no appendix is present, for convenience, we define this category also as a CAP-type.

The shape and size variation of filaments and stopples, and marking the shields allowed us to distinguish if different CAPs were found in the same female in consecutive observations. Obviously, consecutive observations of no CAPs did not inform us about mating events happened between the two observations, since CAPs can be lost without replacement. Therefore, no CAP does not inform on virginity.

3.5. Measurements

All measurements were done on live specimens. We measured the length, height and width of the shields twice subsequently with callipers with thinned jaws for better access, and used the means of the two measurements. Length was the longest distance between the anterior tip and the ventral posterior end of the shield (**Figure 5**). Height was the largest distance from the ventral posterior tip of the shield until the depth gauge hit the female’s body (**Figure 5**). The latter measurement point could not be defined in a more accurate way, due to the large variation of the shape and relative position of the shield on the female body. Width was the largest distance between the left and right lateral walls of the shield at the hind rim (posterior end) of the shield when the callipers had a firm grip on the surface, cca. one mm to the anterior direction at the hind rim (**Figure 5**). We also took photo macrographs from both lateral and posterior views of the shields, as well as from different angles on the area around the copulatory opening (ostium bursae) of non-shielded females (**Figure 5**). We shot dozens of photographs on the ostium bursae at each session on the same female, in order to clearly distinguish (i) filaments from stopples, quite easy with a little practice in scrutinising all the photos and (ii) small CAP shapes to evaluate if a mating attempt likely occurred between consecutive captures. This informed us on whether a female was bearing a CAP or not. Furthermore, it provided a minimum estimate on within-individual small CAP-replacements based upon unique CAP-morphology. Although we cannot claim that consecutive small CAPs with a similar shape on the same female were really the same CAPs, differences in shape signal subsequent mating events. CAPs are made of a durable, hard, flexible material, far the hardest of anything in the female body. In order to change its shape, a force specifically exercised on the CAP by a similarly hard material is required. We did not observe any alterations in shape or size other than the changes caused by males on the sphragides.

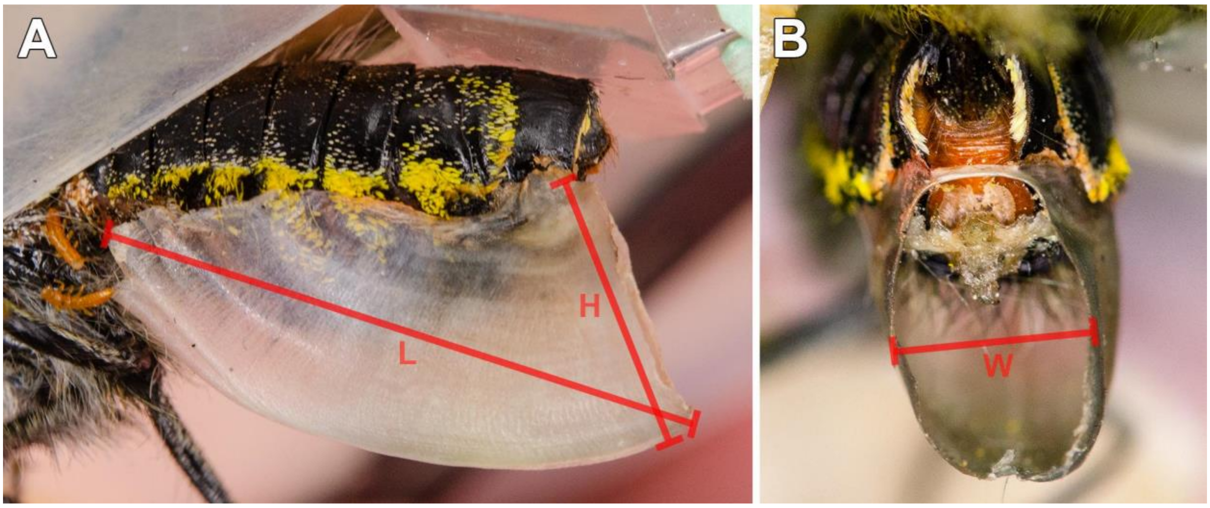


Figure 5. Measurements on the shield of the sphragis. Length (L) was the longest distance between the anterior tip and the ventral posterior end of the shield (A). Height (H) was the largest distance from the ventral posterior tip of the shield until the female’s body (A). Width (W) was the largest distance between its lateral walls (B) measured cca. 1 mm to the anterior from the hind rim along the bar denoted H (A). Photos were taken by JK.

Since matings were scarcely observed, we could associate body size to the CAPs produced only for females. Thorax width was measured at first capture, then we attempted to recapture all marked individuals to repeat thorax width measurements every third day. It was measured twice at each occasion with callipers to 0.1 mm (**Figure 6**B). Thorax size is related to the actual amount of muscles important for butterfly flight (Stjernholm et al. 2005). In Clouded Apollos, thorax width shrinks over an individual’s life with declining body mass (i.e. phenotypic senescence, Pásztor et al. 2022), thus both are related to the actual quality of an individual.

We also recorded three scaled photographs from the anterior view of the head at first capture, then from these photos we measured head width, the largest distance between the lateral edges of the eyes, the widest part of the butterfly head, twice from each photograph (**Figure 6**C). Measurements on photographs were done with Fiji - ImageJ (Schneider et al. 2012). Later in the analysis, the mean of all six measurements was used for each individual. Head width is considered a proxy of body size in insects (Dahlsjö 2016, Hagen & Dupont 2013), including Lepidoptera (Mo et al. 2013), associated with fecundity (Schmidt & Blume 1973). Since it does not change over adult life, it reflects an age-independent quality of an individual.

Other body sizes, such as body mass, wing length (Pásztor et al. 2022) and proboscis length (Szigeti et al. 2020) were also measured (see detailed methodology therein), however these were omitted from the results (see **Appendix 1**) due to their mostly non-significant, uncertain and non-interpretable effects.

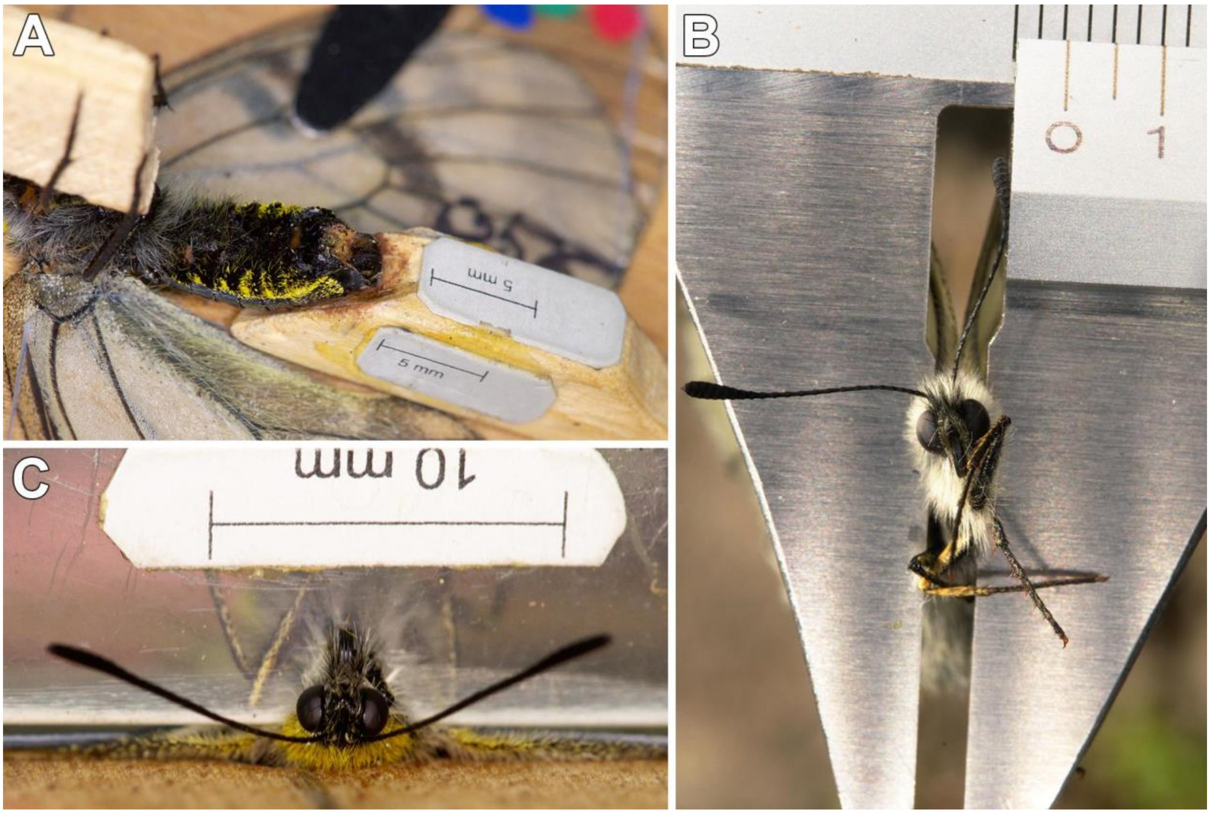


Figure 6. A female Clouded Apollo fixed for genitalia photography (A), thorax width measurement with callipers in a male (B), head width photography in a female (C). Photos were taken by JK.

3.6. Photography

Photo macrographs on mate-guarding devices, i.e. CAPs, female genitalia as well as heads were captured on live individuals fixed on a small board for easy handling. Butterflies were laid on their backs, with wings in a relaxed position and covered with plastic plates, then clipped to the board. The board had a small depression for the dorsal thorax to fit in, in order to prevent tension in wing muscles (**Figure 6**A). Photographs were taken with a Nikon D7000 camera mounted on a tripod, with either a 60mm f/2.8G ED AF-S Micro NIKKOR or a Sigma 105mm f/2.8 EX DG OS MACRO lens.

3.7. Dissection

Although the general anatomy of ditrysian genitalia is well-known (e.g. Orr 1988, 1995, Scoble 1992), we dissected a male and a female Clouded Apollo and illustrated their genitalia to present a comprehensive map on how parts of the genitalia are related to mating and the sphragis position on the female. First, we removed the abdomens of the specimens and removed the shield from the female abdomen by softening it in NH_3. Then we softened the abdomens of both specimens by boiling in 10% KOH solution for 15 minutes. The specimens were captured at Leánykúti-rét in the Visegrádi-hegység in 2007 and killed by pressing the thorax.

3.8. Ethical Note

The Clouded Apollo is a robust butterfly species (Orr 1995), we did not observe any injuries caused by our protocol (measurements, photography and individual marking). All butterflies were released at the site of capture after the measurements and photography. Our field work was licensed by the Hungarian Nature Conservation Authorities: KTVF: 31430/2014.

Dissection was licensed under the conservation grant NKFP (The origin and genesis of the fauna of the Carpathian Basin: diversity, biogeographical hot spots and nature conservation significance; 3B023_04; 2005−2007).

Most photographs were taken on live specimens, except in **Figure 11**H–I on a sphragis we removed and in **Figure 29**E on *Parnassius davydovi*, Churkin 2006: coll: Kirghizstan, Kazarman vic., Moldo-Too 1550, Naryn riv. 2016-08-07, leg: Sochivko, det: Vodjanov. Bought on eBay, deposited at the University of Veterinary Medicine Budapest.

3.9. Statistical analyses

We evaluated all data in the R statistical environment (version 4.2.3, R Core Team 2023). We plotted annual CAP-type event histories (Carey et al. 1998, 2006) to present seasonal CAP-variation across females and years. For plotting, we used the package 'ggplot2' (version 3.4.2; Wickham 2016).

We analysed the annual variation in shield dimensions using ANOVA with year as an explanatory variable and compared means among years with Tukey-tests, with the 'glht' ('multcomp' package, 1.4-20; Hothorn et al. 2008) and the 'cld' ('lsmeans' package, 2.30-0; Lenth 2016) functions. Since females might bear different sphragides consecutively during their lifetime, one sphragis replacing the other, repeated observations may occur, but due to the low number of repeated observations (only eight out of 348 females bore a second sphragis; see the comparison of the models in **Appendix 2**), we did not apply mixed-effects models for this part of the analyses. We also performed correlation tests to show whether there is an association among shield dimensions, using the 'chart.Correlation' function ('PerformanceAnalytics' package, 2.0.4; Peterson & Carl 2020).

3.9.1. Multistate survival model

A semiparametric multistate survival model (Putter et al. 2007) was applied to assess transition risks between the consecutive observations of individual females. Briefly, multistate survival models quantify time-dependent transition probabilities between well-defined states of individuals. The hazard in a multistate survival model is approximately the probability of transition from the current state to a subsequent state during unit time, one day in our model. The hazard depends on the actual follow up time when the transition happens. There are unique hazard submodels for each possible transition between states. In a semiparametric multistate model, the hazards are defined as a product of a nonparametric time-dependent function of a general form and a fully parametric multiplicative expression. This expression depends on certain risk factors or covariates and the corresponding relative risks being the parameters of the model. Relative risks between 0 and 1 indicate a negative effect, values greater than 1 indicate a positive effect with respect to the reference category.

Similar annual patterns in CAP-frequencies (see 4.1.6. CAP-variation section in the Results) allowed the analysis with the years pooled. For the multistate analyses, we distinguished female states as no CAP, small CAP, shield, and disappeared (i.e. the female from the population). We use the term female state only for modelling and discussing model results. Note that this term includes CAP-types, the term we use to describe the mate-guarding devices (or their absence), but is not equivalent with that. We use the term transition for changes among female states, including if a state is followed by the same state within a female (e.g. a small CAP followed by a *different* small CAP). Matings between two consecutive no CAP observations were not detected, i.e. no transitions could be found between two no CAPs. The disappearance of a female is referred to as disappeared, because it might mean death, emigration or that we simply failed to further observe the butterfly. A previous mark-release-recapture study on this population between 2016 and 2019 using Jolly-Seber models estimated that ~90% of the individuals in this relatively closed population had been captured at least once in each year (Zorkóczy 2020), suggesting that individuals present in the population were mostly detected.

Where no CAP was not the first observed female state (344 females out of 492), we added one day of no CAP to female life preceding first detection, since females start their life virgin, as no CAP. However, most females are first detected with CAPs, i.e. when already mated, hence we assumed that they had mated very soon after emergence. We pooled the CAP-types filament and stopple into small CAP, since these are much smaller than the shortest shields, therefore we assume that, compared to shields, male investment and their capacity to prevent future matings are similarly small. This was also necessary due to the low number of observations per the three CAP-types, thus this made the model fit better and allowed simple interpretation. Small CAPs and shields were sometimes replaced with the same CAP-type in an individual between two consecutive observations. However, multistate models cannot recognise this pattern of change as a state transition. To overcome this problem, we introduced dummy states, e.g. a shield could transition to the dummy then the dummy into another shield, with the time ‘spent’ in the dummy state closing to zero. This resulted in slightly different frequencies of model transitions compared to the number of actual replacements that we observed in the field.

We intended to evaluate the differences in risks of small CAP and shield losses. However, we found interactions between relative risks and time in our multistate model. The complexity of the interaction model did not allow to reliably interpret the comparisons of the Cox method. Therefore, to simplify the evaluation, we computed 99.16% confidence intervals around the cumulative hazards of the small CAP and shield losses. This allows for multiple comparisons of the small CAP and shield loss risks at 5% adjusted significance level, using Bonferroni's method. All three transitions from small CAP were compared to the shield to small CAP (3 comparisons) and to the shield to shield transitions (3 comparisons). For better visualisation, we transformed transition hazards from the model as ln(hazard × 10 + 1) for the cumulative hazards plot.

3.9.2. Submodels

Within the framework of this complex model, we fitted nonparametric cause-specific hazard functions to each transition between states to estimate instantaneous transition risks at each time point measured since the onset of the follow-up. The package 'mstate' (version 0.3.2; de Wreede et al. 2011) was applied to fit the multistate survival model. We fitted proportional hazard submodels to investigate the differences in relative risks among transitions starting from the same states (e.g. shield to shield and shield to small CAP). To account for time-dependent changes in transition risks, we included interaction terms between time and transitions.

Each body size variable, shield length, as well as adult sex ratio were assigned to different submodels, separately focusing on the relative risks of transitions associated to each of these covariates. To use covariates in these submodels, we had to transform some of the variables. Based upon **Figure 27**, seeking for the best separating threshold between the lost and the permanent shields, we converted the continuous shield length (the most varying shield dimension) to a binary variable; below the 90th percentile of lost shields we defined the shields as short, above it, as long. The 90th percentile threshold allowed to separate most lost shields from most permanent shields. We used this threshold to define long and short shield categories in the multistate model. This binary variable allowed a simple interpretation of the results. To provide an estimate of the actual condition of a female unrelated to its natal body size (i.e. size at emergence), we used the natural logarithm of the thorax width to head width ratio, i.e. ln(thorax width) − ln(head width), calculated for every time point of the transitions. We used the exact or the closest (±3 days) thorax width values to the day of the transition. We calculated shield-to-CAP ratios for each day, the number of shields observed per day divided by the total number of daily CAPs. We also calculated daily adult sex ratios (ASR; Kvarnemo & Ahnesjö 2002) and used them as either a continuous (time-series analysis; see 3.9.3) or a binary variable (multistate model; for simple interpretation). In the latter the population was considered female-biased below, and male-biased above 0.5. Daily ASR (the number of males / the number of males + females) involved all alive individuals on a given day in the population deduced from the mark-recapture encounter histories and was calculated for each day of the flight period, regardless if a specific individual was actually observed or not on the given day.

In further submodels involving covariates, we included (i) the binary shield length as factor, or (ii) the continuous thorax width to head width ratio (female's actual condition controlled for natal size), as well as head width (natal size), or (iii) the binary adult sex ratio as factor. In the submodels containing thorax width to head width ratio or shield length, data were grouped by the individual identification numbers – applying the ‘cluster()’ term in the R code of the multistate survival model – to obtain proper marginal estimates (Therneau & Grambsch 2000), because females were measured multiple times and could bear multiple shields during their lifetime. Relative risks (RR) of covariate effects modifying baseline transition hazards were estimated. The nonlinear effect of thorax width to head width ratio was accounted for by including the squares and cubes of this variable in the submodel. These additional transformed variables proved to be non-significant and thus eliminated from the final submodels. The hazard submodels were fitted using a stratified Cox proportional hazards model (Therneau & Grambsch 2000) with time-dependent covariates applying the R function coxph from the package ’survival’ (version 3.5-5; Therneau 2023). We used the Breslow method when compiling the submodels. To test the proportional hazards assumption, we used Schoenfeld residuals (Therneau & Grambsch 2000). Martingale residuals and deviance residuals (Therneau & Grambsch 2000) were also inspected to detect potential influential points causing bias.

3.9.3. Time-series analysis

To investigate the time-dependent relationship between daily shield-to-CAP ratio and daily adult sex ratio (ASR) as a continuous variable, we performed a time series analysis. We excluded (i) days where ratios were equal either to zero or one (when only shields or small CAPs, or males or females were present in the population on a specific day; these occurred at the beginning and at the end of the flight periods when the number of individuals was low), and (ii) influential points (days 2015-05-29 and 2015-05-25), based on scatterplots, as well as (iii) the entire year 2020 due to the very low number of butterflies and small CAPs (**Figure 7**, **Figure 8**). This resulted the best fitting model. Then we concatenated the time dependent data of subsequent years between 2015 and 2019, and used the cross-correlation function (CCF; package ‘astsa’, version 2.0; Stoffer & Poison 2023) to compute the correlation between the time series of the two ratios. We transformed these using natural logarithm, then calculated the difference between subsequent daily values as $\ln(\text{ratio}[t]) - \ln(\text{ratio}[t - 1])$. This was necessary to be able to investigate the percentage change in ratios between days, the model providing the best fit to these data. In the CCF, the three-day lag provided the largest absolute value of the correlation coefficient (**Figure 9**). We fitted a generalised linear mixed effects model (GLMM, ‘lme’ function; package ‘nlme’, version 3.1-162; Pinheiro & Bates 2000), for the logarithm of daily shield-to-CAP ratio using the three-day time lag of the logarithm of adult sex ratio as the explanatory variable, assuming first order autoregressive (AR1) residuals. This means that the change in shield-to-CAP ratios followed the adult sex ratios with a three-day lag. The year of observation was included as a random intercept. Based on this model, we estimated elasticity (Sydsæter et al. 2016) between shield-to-CAP ratios and the adult sex ratios three days earlier.

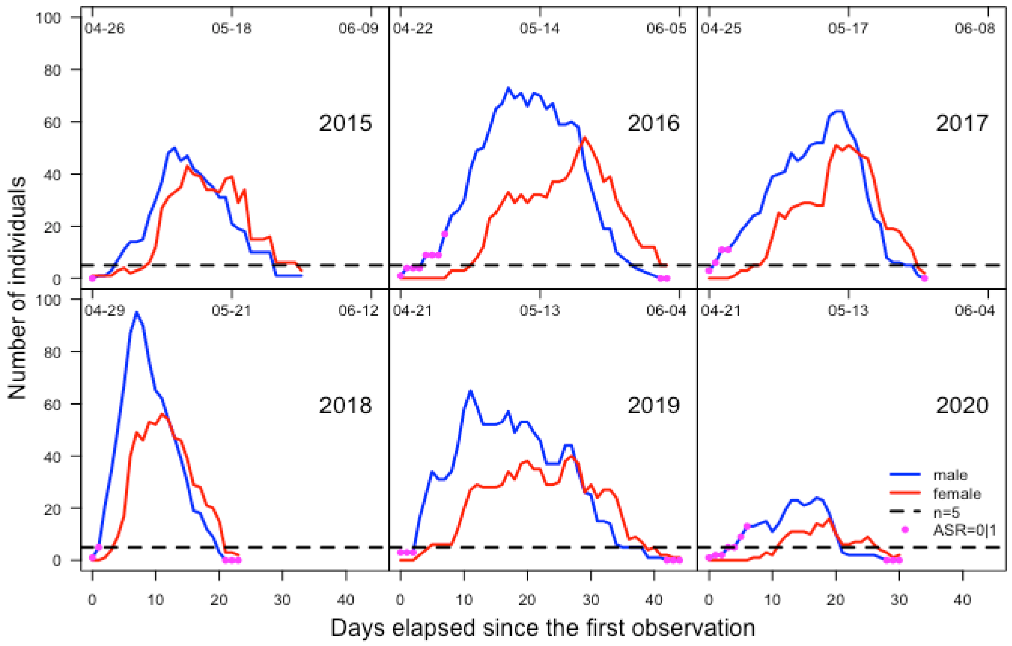


Figure 7. The daily number of Clouded Apollo males (blue lines) and females (red lines) during the flight period from 2015 to 2020. The top horizontal axes show dates; note the differences across years. Black dashed lines show the threshold where the number of individuals equals 5. Magenta dots mark days where adult sex ratios (ASR) were equal either to zero or one.

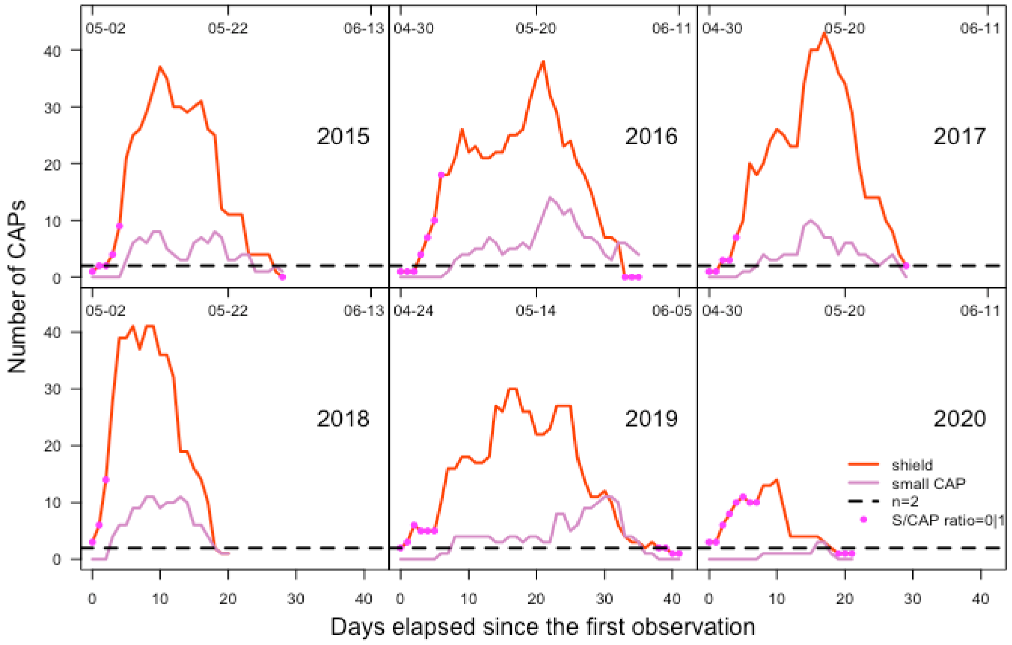


Figure 8. The daily numbers of Copulatory opening APpendix (CAP) types: shields (orange lines) and small CAPs (purple lines) during the flight period from 2015 to 2020 in Clouded Apollos. The top horizontal axes show dates; note the differences across years. Black dashed lines show the threshold where the number of CAPs equals 2. Magenta dots mark days where shield-to-CAP ratios were equal either to zero or one.

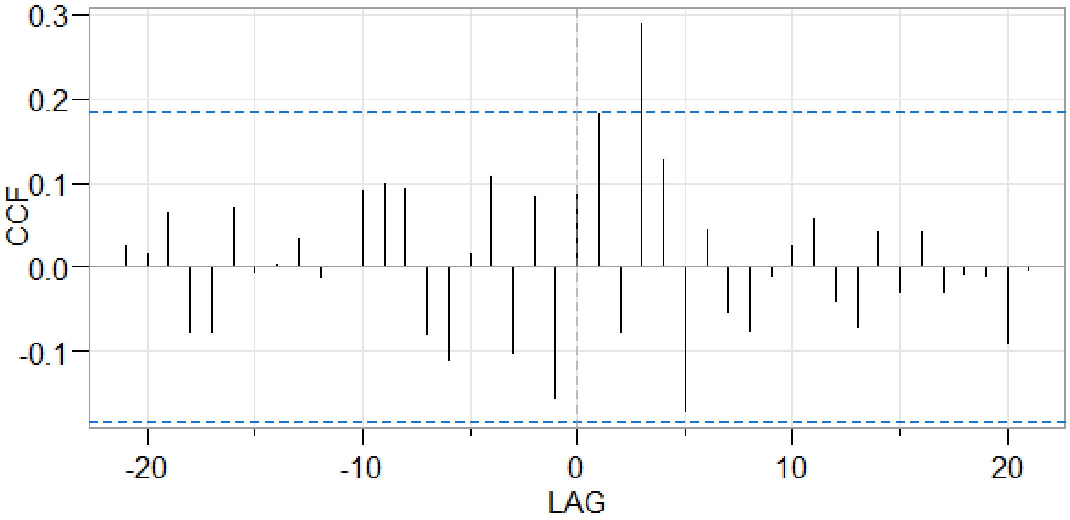


Figure 9. Cross-correlation function (CCF) computed between the time series of the adult sex ratios and shield-to-CAP ratios in Clouded Apollos. The blue dashed lines show the 95% confidence interval for the correlation coefficients. LAG shows the number of days.

4. Results

4.1. The description of the reproductive system in Clouded Apollo butterflies

Published as: Gór, Á., Fónagy, A., Pásztor, K., Szigeti, V., Lang, Z., & Kis, J. (2023). Facultative male investment in prolonged mate-guarding in a butterfly. Behaviour, 160(6), 515-557. https://doi.org/10.1163/1568539X-bja10219

An abbreviated version was also published in Hungarian as: Gór, Á., Fónagy, A., Pásztor, K., Szigeti, V., Lang, Z., & Kis, J. (2023). Változó hím befektetés a nőstény további párosodását akadályozó képletekbe kis apollólepkénél (Parnassius mnemosyne (LINNAEUS, 1758)). Állattani Közlemények, 108(1-2). https://doi.org/10.20331/AllKoz.2023.108.1-2.3

Between 2015 and 2020 we observed a total number of 492 Clouded Apollo females. The duration of the flight periods and the number of females varied considerably across years. The longest was in 2019 with 45 days, the shortest was in 2018 with 26 days. The most females were present in 2018 with 116 individuals, the lowest number of females was in 2020 with 34 individuals, when the total number of Clouded Apollos dropped drastically (**Table 1**).

4.1.1. Terminology

We distinguished three morphologically distinct types of male-produced mate-guarding devices as externally observable appendices attached to the female copulatory opening (ostium bursae; **Figure 10**E, F, **Figure 11**B–G) during mating. We call these Copulatory opening APpendices, or CAPs. Additionally, females may lack an externally observable CAP, i.e. they have a clear sinus vaginalis (the shallow pocket in which the copulatory opening is situated; **Figure 10**E, **Figure 11**A; Carvalho et al. 2017, Scoble 1992). We denote this female state as ‘no CAP’, as opposed to ‘CAPped’. CAPs either cap or at least impede access to the copulatory opening, and all types are easy to unambiguously identify with external scrutiny (summarised in **Table 2**, photographic examples in **Figure 11**A–I).

Table 2. Copulatory opening APpendix (CAP) definitions in Clouded Apollo butterflies. ‘General term’ refers to terms widely used in the literature. Note the overlaps among our terms and the general terms. Assumed male costs as well as effectiveness in mate-guarding increase with CAP size and structural complexity, from no CAP to shield.

CAP type		general term		definition	assumed	
					male costs	role in guarding
no CAP		mating plug		– no appendix can be observed externally in the copulatory opening (note: does not inform on virginity; internal plug might occur)	size structural complexity ↓	no / very low
filament	small CAP			– thin, elongated, thread-like, extending from the copulatory opening – the sinus vaginalis of the opening is not filled; may be short, hardly visible		low
stopple			sphragis	– compact, at least partially fills the sinus of the copulatory opening – may extend well over the copulatory opening and cover the sinus entirely		medium
shield				– a sheet warped around and attached to the stopple with lateral bridges – much larger than the stopple; may cover most of the ventral abdomen		high

The smallest in volume and less structured CAP is the (i) filament, a thin, elongated, thread-like appendix, extending from the copulatory opening; the sinus of the opening is not filled. Its length may vary, including very short, hardly visible filaments (**Figure 21**A); a ~10× magnifier is needed for most observers. A (ii) stopple is noticeably larger and fills the sinus of the copulatory opening at least partially, and it may bear 1–3 threads similar to the filament (**Figure 12**A, B). The stopple is compact and amorphous, and may extend over the copulatory opening covering it entirely; a ~10× magnifier may be needed. We refer to the filament and the stopple together as a ‘small CAP’ for convenience if distinguishing these two is not convenient when compared to the much larger and more structured shield. A (iii) shield is a sheet warped around and attached to the stopple with lateral bridges (**Figure 10**G–I, **Figure 11**E–I), and is well-visible for the naked eye. This suggests that a stopple is formed first, and the male may develop it further by building a shield around the stopple. A shield may extend over the ventral side of the abdomen, often covering it and may stretch almost to the thorax, albeit in a few cases it is too short to stretch over the abdomen (**Figure 22**A). A thread similar to the filament is often observed on a stopple, even when it is shielded (**Figure 12**C). The shielded stopple approximately corresponds to the term of the hollow type of large sphragis (category 4a, sensu Carvalho et al. 2017, see also Orr 1995). CAPs do not block egg laying in Clouded Apollos (ditrysia; **Figure 10**E, F, I; Scoble 1992). We use the term ‘shield’ to emphasise the differences of the three CAP-types or refer to the shields’ dimensions.

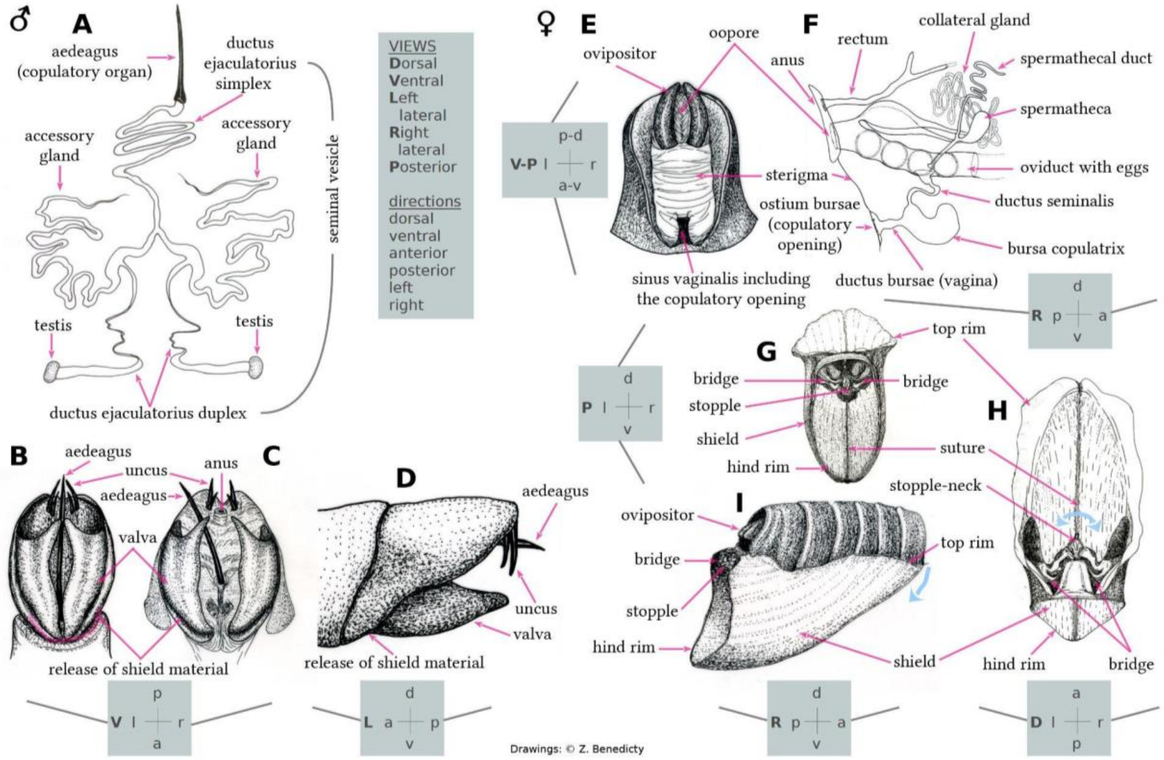


Figure 10. Anatomy of the genitalia and the sphragis in Clouded Apollos. Internal (A) and external male genitalia (B–D). External (E) and internal female genitalia (F). The structure (G–H) and position on the female (I) of the complete, large, hollow sphragis. The seminal vesicle consists of the paired ductus ejaculatorius duplex and the unpaired simplex (A). The valvae (singular: valva, in Clouded Apollos, a ventral pair of sclerotised claspers of the male genitalia) and the unci (singular: uncus, in Clouded Apollos, two posterior pairs of sclerotised claspers of the male genitalia) are closed (B) and open (C) with the extended male copulatory organ (aedeagus). The position of the ovipositor and the externalised sterigma bearing the sinus vaginalis in which the female copulatory opening is situated (E). Female internal genitalia (F). The separate channels for copulation and oviposition in Ditrysia allow the permanent capping of females without blocking egg laying (E, F, I). The curved, blue arrow shows the direction in the median plane of moving the anterior tip of the shield away from the female abdomen by the male in order to loosen the stopple in the vagina (I). The other curved, blue arrow shows the potential rotation of a sphragis around the stopple-neck in the frontal plane, when the sphragis is loosened in case of a removal attempt (H). Further details are described in the text. Drawn by Z. Benedicty, dissections by A. Fónagy, designed by JK.

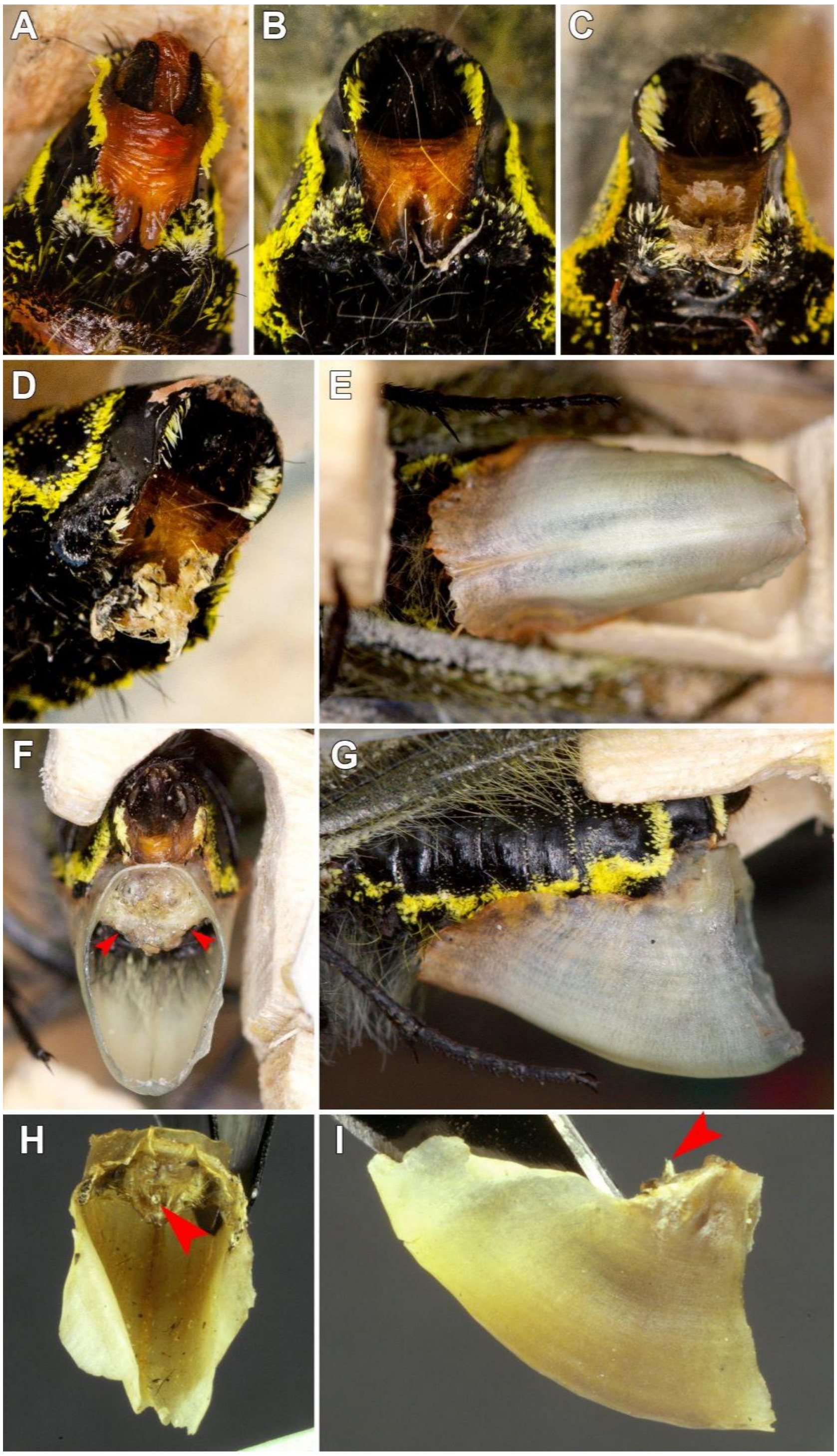


Figure 11. Copulatory opening APpendix (CAP) types in Clouded Apollos. No appendix seen in the copulatory opening (no CAP; posterior-ventral view; A). Filament; note that the sinus vaginalis is not filled (posterior-ventral view; B). Stopples on different females (note that the sinus vaginalis is fully covered; posterior-ventral & left-posterior views, respectively; C–D). The same shield from ventral (E), posterior (F) and lateral (G) views; the arrows point to the lateral bridges that connect the shield to the stopple (F). Shield artificially removed from a dead female; dorsal (H), and left view (I). The arrows (H–I) point to the stopple-neck where it was fastened in the copulatory opening. The stopple-neck was torn when the shield was removed from the female. Photos were taken by JK.

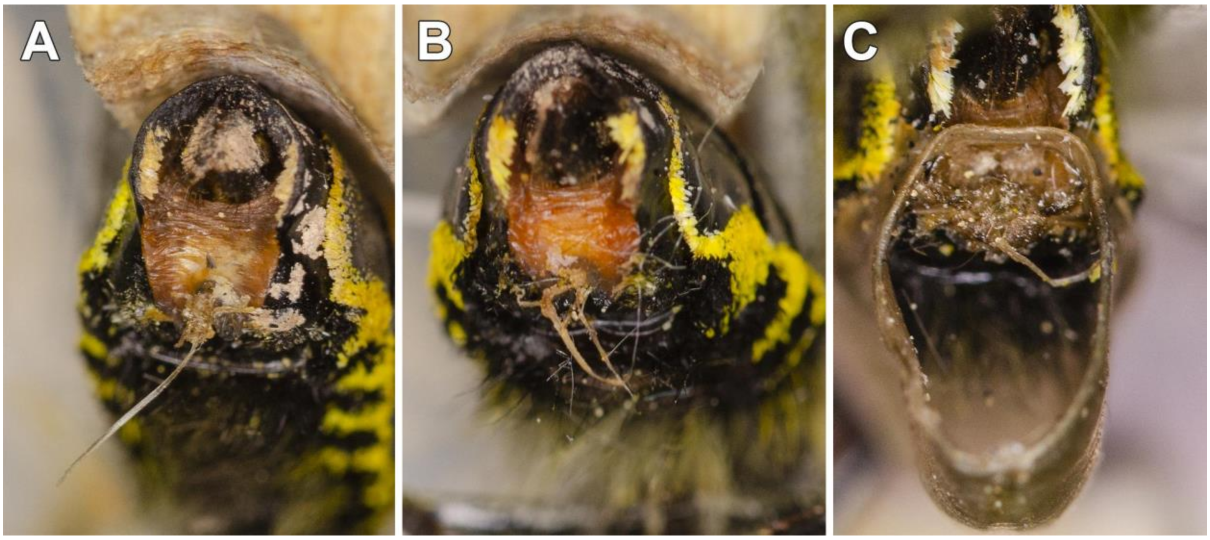


Figure 12. Threads similar to the filament can often be found on stopples in Clouded Apollos (A–B), even if a shield was built around the stopple (C). The two filaments might indicate two subsequent mating attempts (B). Photos were taken by JK.

4.1.2. The Hungarian terminology

In the second, Hungarian publication we introduced the term ‘PárzóNyílás-Függelék (PNyF)’ for Copulatory opening APendix (CAP). Within that, we used the term ‘nincs PNyF’ for no CAP, ‘filamentum’ for filament, ‘plomba’ for stopple, ‘pajzs’ for shield and ‘szfrágisz’ for sphragis (**Table 3**). In Hungarian literature, the traditional term for the sphragis is ‘erényöv’. Although it describes its function well, we find it to be a too anthropogenic term, so we recommend using the term ‘szfrágisz’ instead.

Table 3. English terms and their Hungarian equivalents describing the devices found in Clouded Apollo butterflies.

English terms	Hungarian equivalents
Copulatory opening APendix (CAP)	*PárzóNyílás-Függelék (PNyF)*
no CAP	*nincs PNyF (nPNyF)*
filament	*filamentum*
stopple	*plomba*
small CAP	*kis PNyF (kPNyF)*
shield	*pajzs*
sphragis	*szfrágisz*

4.1.3. Mating behaviour

We observed a few males and females that mated more than once during their lifetime. Clouded Apollo males mate without courtship (as in related species; Kawagoe et al. 2001, Matsumoto et al. 2018, McCorkle & Hammond 1985, Orr & Rutowski 1991, Orr 1988, 1995, 2002). We observed that when a patrolling male notices a female, engages it, then tries to grasp the female's abdomen with its legs to keep it in position. Males often try to engage other males (or mating pairs, sometimes ending their copulation), but ignore freshly emerged females yet with an orange tint in their wings that would later whiten (in a few hours) with maturation (**Figure 1**). Once grabbing the female, the male tries getting into the 'mating triangle' position (**Figure 13**A–C), characteristic to most butterflies, the pair facing the opposite direction with the posterior tip of the abdomen attached. The female often carries the male around until it finds a suitable place, usually in the dense grass, while the male hangs on the female mostly motionless. Occasionally the female crawls upwards on plants, around 20–30 cm above ground level. Matings may last from a few dozens of minutes up to several hours, during which males may form one of the three types of CAPs. Matings started in the afternoon may last overnight, probably due to the freezing of the process when the mates cool down. The shield originates from around the male valvae (singular: valva, in Clouded Apollos, a ventral pair of sclerotised claspers of the male genitalia; **Figure 10**B–D, **Figure 14**A–C) and hardens as it is made, being formed towards the end of the mating. We observed a few times males bearing shields or shield residue (**Figure 14**A–D).

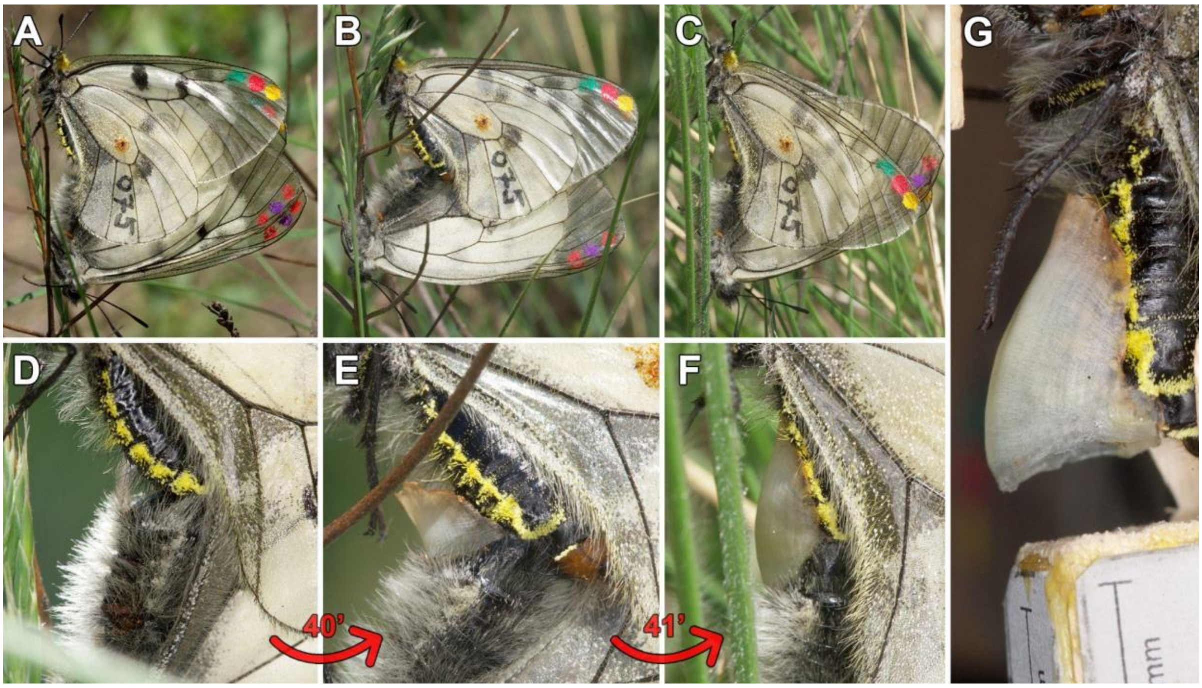


Figure 13. Shield development in a Clouded Apollo pair. The top row shows the couple, A and D 40 minutes prior to B and E, B and E 41 minutes prior to C and F. The bottom row shows close-ups of the couple's abdomens (D–F), the timing paired according to columns. The final form of the produced shield (G). Female wings usually enclose male wings in the 'mating triangle' position. Note that females have yellow scales and both sexes had already been marked with numbers and colour dots prior to this observation. Photos were taken by JK.

Figure 14. CAP-material stuck on Clouded Apollo males. Left-ventral (A) and ventral (B) view of the same shield. Left-ventral view of a half shield on a different male (C). CAP-residue (red arrow) near the unci (see **Figure 10**B–D), around the reach of the tip of the aedeagus (not seen here; D). Photos were taken by JK.

4.1.4. Oviposition

A mated female searches for habitat patches rich in *Corydalis* spp., the larval food plant. Females spend a few minutes crawling the litter or grass in search of a suitable surface to glue their eggs on, and they lay one or a few eggs mostly on litter, grass or bark in one bout, then basking on sunny spots before the next laying bout. Observations suggest that shields may impede oviposition, since they make it difficult for the ovipositor to find a suitable surface. We also found eggs stuck on the shields' outer surface in a few cases (**Figure 15**A–D).

Figure 15. Eggs stuck on shields (A, C, D), or the body (B, D) of shielded Clouded Apollos. The black patches on the shields are our markings. Photos were taken by JK.

4.1.5. CAP-removal

We observed and video-recorded 7 cases of sphragis removals and removal attempts. Males try to grasp the female body with their legs (**Video 4**, 0:00) and the shield with the valvae (**Video 4**, 0:00). They attempt to have a firm grip on the shield by the valvae and unci (singular: uncus, in Clouded Apollos, two posterior pairs of sclerotised claspers of the male genitalia; **Figure 10**B–D, **Figure 30**F; **Video 2**, 0:00), and lift the shield's anterior part (**Figure 10**I; **Video 4**, 0:00). Simultaneously, the valvae slowly press-release-press on the shield to loosen it (**Figure 10**H; angle of rotation; **Video 4**, 0:00; Video 2, 0:00). In the meantime, the sclerotised, sharp-pointed male copulatory organ (aedeagus; **Figure 10**A–D) may pick around the female copulatory opening where the sphragis is fastened to the female (**Figure 16**J, L; **Video 4**, 0:37). When the sphragis is loosened, it may rotate around the stopple-neck (**Figure 10**H; angle of rotation; **Video 4**, 1:11), and the male may try to sever the sphragis by cutting the stopple-neck off the internal plug with the sharp-pointed tips of the valvae (**Video 4**, 0:37, 1:11). After severing the sphragis, the valvae grab and move it away from the female copulatory opening with the movement of the male abdomen (Video 2, 1:08). A successful shield removal is followed by picking at the copulatory opening with both the valvae and the aedeagus (**Figure 10**A–D; **Video 4**, 1:29; Video 2, 1:27; **Video 4**, 0:00) before the triangle-shaped mating position is formed (**Video 4**, 1:44; Video 2, 1:58). Males may abandon the female after a period of an unsuccessful removal attempt (**Video 4**, 0:45). Due to the very low number of observations we had on males giving up, and because we found the couples with the males already attempting to remove the sphragis, we cannot provide reliable estimates on the latency of the males giving up. One observation indicates approximately an hour, but we expect a high variance. Furthermore, in a few females we found holes, ranging from 1 to 5 (**Figure 17**A–E, H), ruptures and creases on shields (**Figure 18**D, H, L, P), hanging and broken shields (**Figure 18**B, F, J, N) that are most likely the results of sphragis removal attempts.

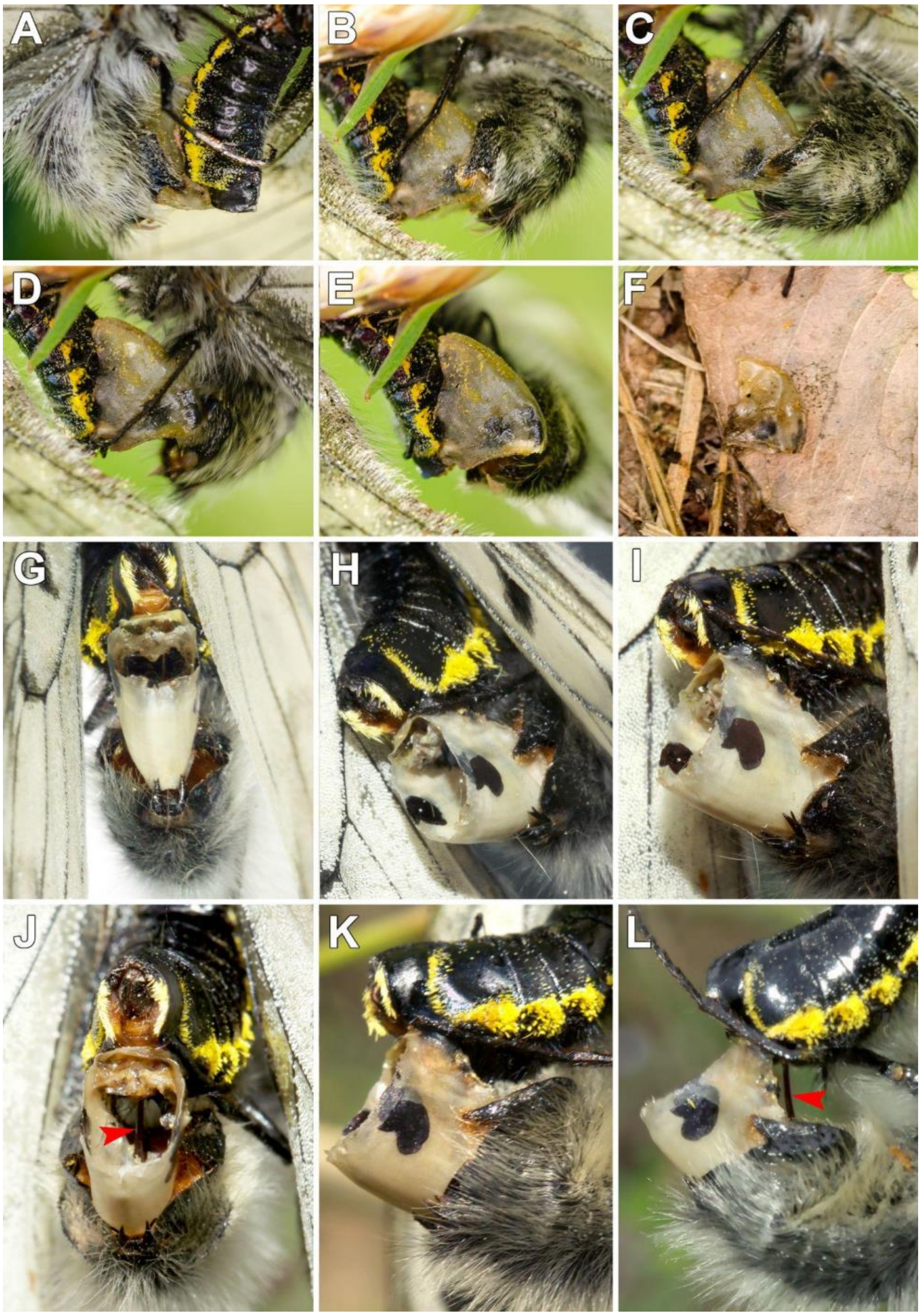


Figure 16. Sphragis removal attempts in Clouded Apollos. Successful removal (A–F). The male holds the female with its legs, while the valvae are holding the shield (A). By bending its abdomen, the male tries to exert a force to lift the anterior end of the shield and move it away from the female abdomen to loosen the stopple-neck in the vagina (B–E; also H, I, K, L; **Figure 11**H, I; **Video 4**, 0:00). The sphragis removed (F; **Video 2**, 2:17). Positions of the valvae and the unci (G–L) during sphragis removal attempts. An unsuccessful (G–I) and a successful (J–L; **Video 3**, 0:00) removal attempt of the same sphragis by two different males, three days apart. Due to deformation, the length of the shield shrunk by 1 mm after the 1st attempt. Note the position of the aedeagus thrusting towards the copulatory opening of the female (red arrows J, L). The black patches on the shields are our markings. Photos were taken by JK.

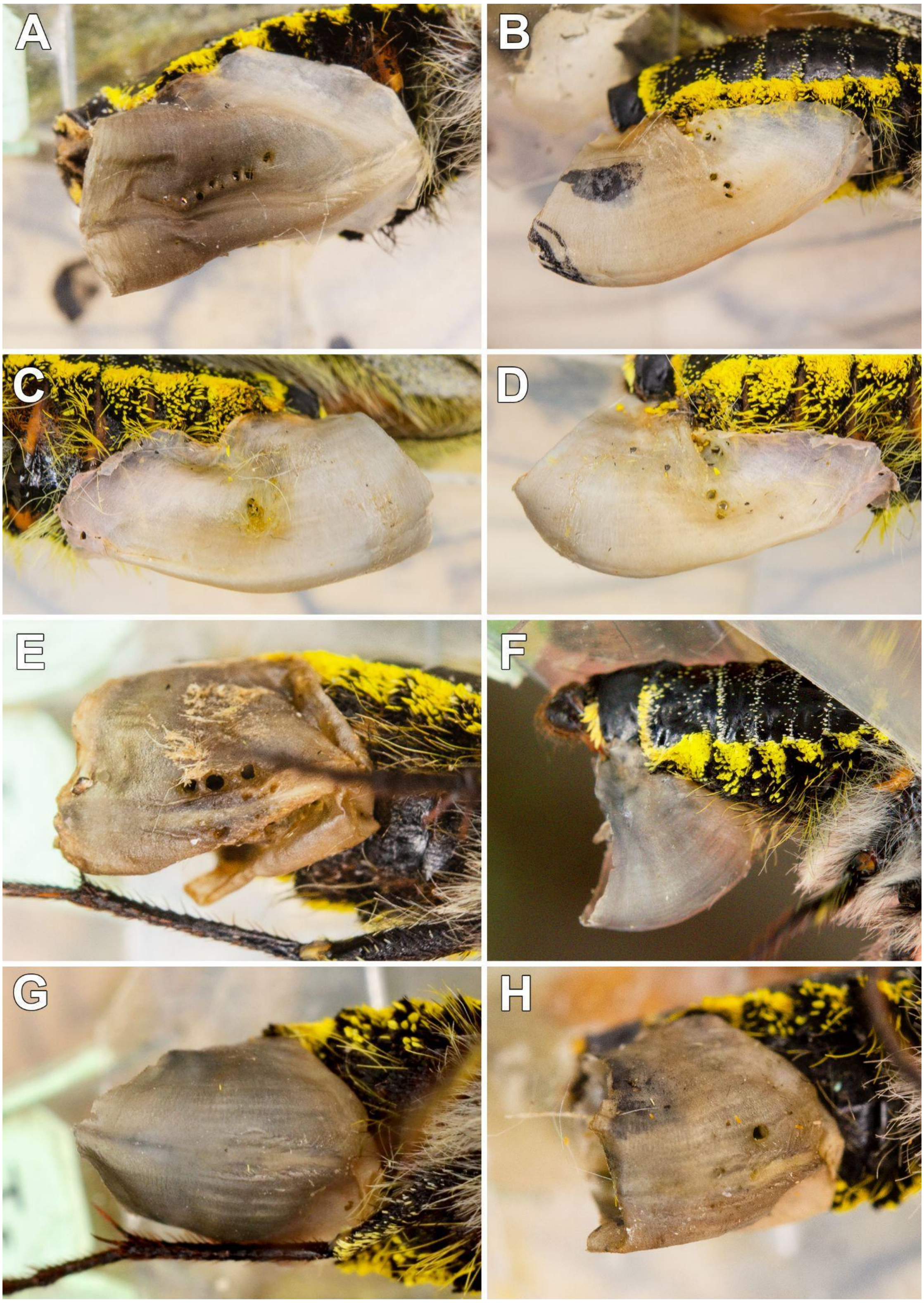


Figure 17. Perforated and deformed shields in Clouded Apollos. Shields are detected as perforated, from different females, except (H) which was perforated after the female had been observed with an intact shield (F–G). Note how the shape of the shield has also changed (G–H). One of the five shields is photographed from both sides (C–D). The black patches on the shields are our markings. Photos were taken by JK.

Figure 18. Torn and deformed shields with creases and ruptures in Clouded Apollos. Photographs taken at the 1st occasion the shield was detected (1st and 3rd column from left to right) and after shield injury was first detected (2nd and 4th column), showing the same 8 shields of 8 different females. The black patches on the shields are our markings. Photos were taken by JK.

Videos 1–4. Video recordings of successful and failed sphragis removals, and failed stopple removal in Clouded Apollo butterflies. Scan the QR code or visit the following link to see the video recordings and the detailed captions. Link: BEH 3812 Videos

4.1.6. CAP-variation

We observed a total of 154 cases of no CAPs, 127 filaments, 120 stopples and 356 shields between 2015 and 2020. The number of the devices varied across years, as well as the annual proportion of shielded females compared to the non-shielded, with a minimum of 67.9% and a maximum of 80.0%. We observed a total of 23 shield losses (**Table 4**), but only 22 of the lost shields were measured.

Table 4. Frequencies of Clouded Apollo Copulatory opening APpendix (CAP) CAP-types over six years. Small CAPs consist of filaments and stopples. The number of females observed with a specific CAP-type is shown in parentheses. We also provide the percentage of shielded out of all females and the number of shields lost. The no CAPs column shows cases when no CAP females were actually observed. A female could be observed with several different or similar consecutive CAPs during its life.

Year	$n_{\text{no CAPs}}$	$n_{\text{filaments}}$	n_{stopples}	$n_{\text{small CAPs}}$	n_{shields}	$\%_{\text{shielded females}}$	$n_{\text{lost shields}}$
2015	18 (18)	20 (20)	21 (17)	41 (29)	61 (57)	67.9	7
2016	31 (31)	31 (22)	32 (23)	63 (35)	62 (62)	70.5	5
2017	26 (26)	21 (14)	16 (15)	37 (22)	63 (62)	71.3	2
2018	30 (30)	30 (19)	20 (17)	50 (30)	87 (85)	80.0	7
2019	35 (35)	23 (15)	27 (17)	50 (25)	58 (57)	68.7	2
2020	14 (14)	2 (1)	4 (4)	6 (4)	25 (25)	74.0	0
2015 – 2020	154 (154)	127 (91)	120 (93)	247 (145)	356 (348)	73.0	23

We measured 356 shields in 348 females between 2015 and 2020. The dimensions varied considerably among shields in length (min – max: 3.78 – 13.00 mm; mean ± SD: 9.47 ± 1.36 mm), height (min – max: 2.35 – 5.23 mm; mean ± SD: 4.29 ± 0.43 mm) and width (min – max: 1.98 – 3.93 mm; mean ± SD: 3.21 ± 0.25 mm) (**Table 5**). The differences between minimum and maximum sizes were remarkably large compared to the mean (length range 9.22 mm; 97% of the mean; height range 2.88 mm; 67% of the mean; width range 1.95 mm; 61% of the mean). Shield length did not vary among years, but height and width varied moderately (**Figure 19**). Shield length and height were positively, height and width negatively correlated albeit both correlation coefficients were small, and length and width were not significantly related (**Figure 20**). We did not separate years due to their negligible effects.

Table 5. Annual variation in shield length, height and width with minimum, maximum, mean and SD [mm].

	Length				**Height**				**Width**			
Year	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
2015	4.200	11.625	9.422	1.452	2.350	4.875	4.101	0.512	2.800	3.700	3.249	0.166
2016	4.000	12.350	9.367	1.580	2.475	4.925	4.124	0.461	2.625	3.850	3.352	0.261
2017	5.700	11.925	9.392	1.299	2.725	5.100	4.297	0.413	2.475	3.700	3.248	0.225
2018	3.775	13.000	9.443	1.343	3.250	5.025	4.391	0.357	1.975	3.650	3.122	0.253
2019	6.125	12.150	9.551	1.179	3.650	5.225	4.481	0.326	2.525	3.925	3.194	0.237
2020	7.650	12.750	9.892	1.227	3.900	4.850	4.320	0.261	2.675	3.400	3.038	0.207
2015 – 2020	3.775	13.000	9.466	1.364	2.350	5.225	4.288	0.427	1.975	3.925	3.214	0.246

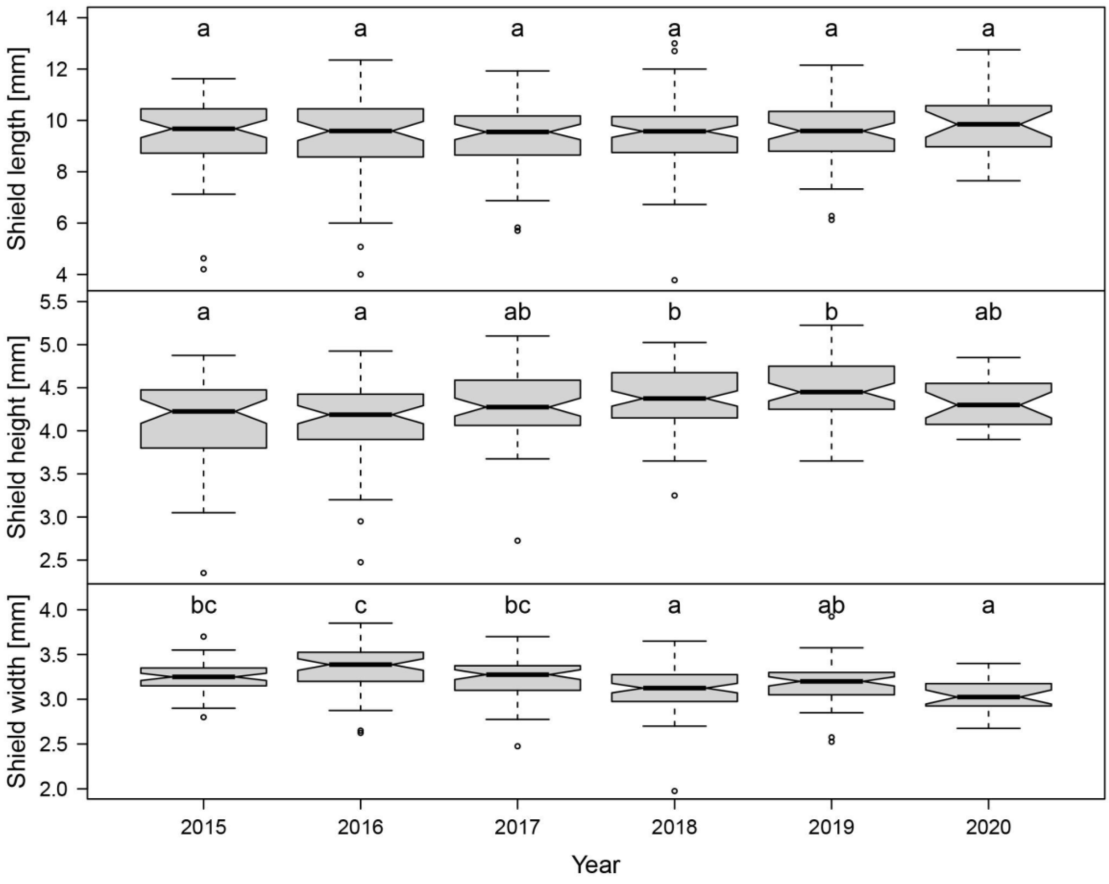


Figure 19. Shield length, height and width [mm] variation across years. Boxplots depict the interquartile range with 95% confidence intervals (notches) of the median (horizontal, central line). Whiskers include the range of distribution without outliers. Outliers were defined as data beyond 1.5 × the interquartile range above the upper quartile and below the lower quartile. Different letters above the boxes show significantly different years (Tukey HSD test).

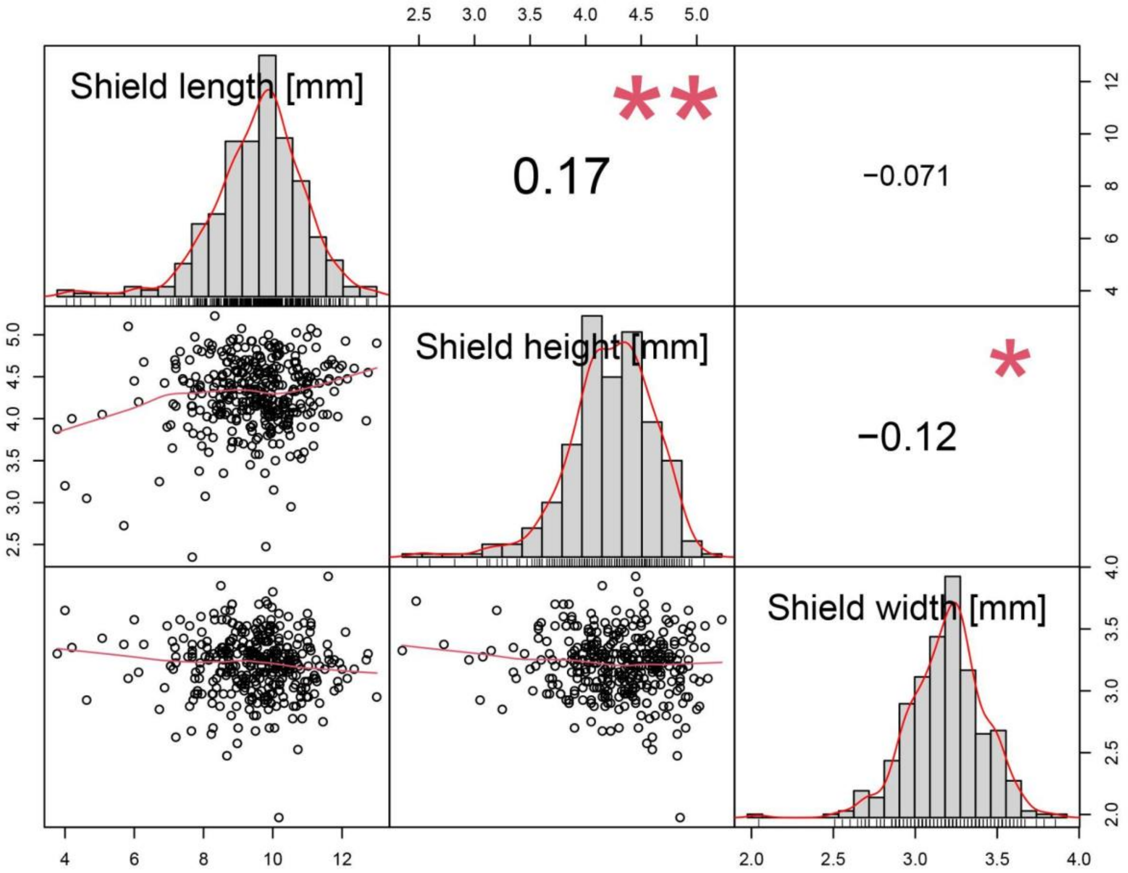


Figure 20. Distributions and pairwise correlations (*Spearman's ρ*) of shield dimensions [mm] in Clouded Apollo butterflies. The diagonal shows data distribution. Bottom left of the diagonal: bivariate scatter plots with loess-fitted line (Locally estimated scatterplot smoothing). Top right of the diagonal: correlation coefficients with significance levels: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n = 356.

During field observations, we found considerable variation in filament (**Figure 21**A–D), stopple (**Figure 21**E–H) and shield shape, size, colour (**Figure 22**A–I) and deformations (**Figure 18**B, D, F, H, J, L, N, P). Filaments ranged from short (**Figure 21**A) and often thick sticks to very thin and long threads (**Figure 21**B–D), while stopples ranged from small external plugs to large seal-like devices covering the whole sinus vaginalis (**Figure 21**E–H). Shield colour ranged from ivory to greyish/dark with rose/yellowish/greenish perfusion and from transparent to translucent (**Figure 22**A–I).

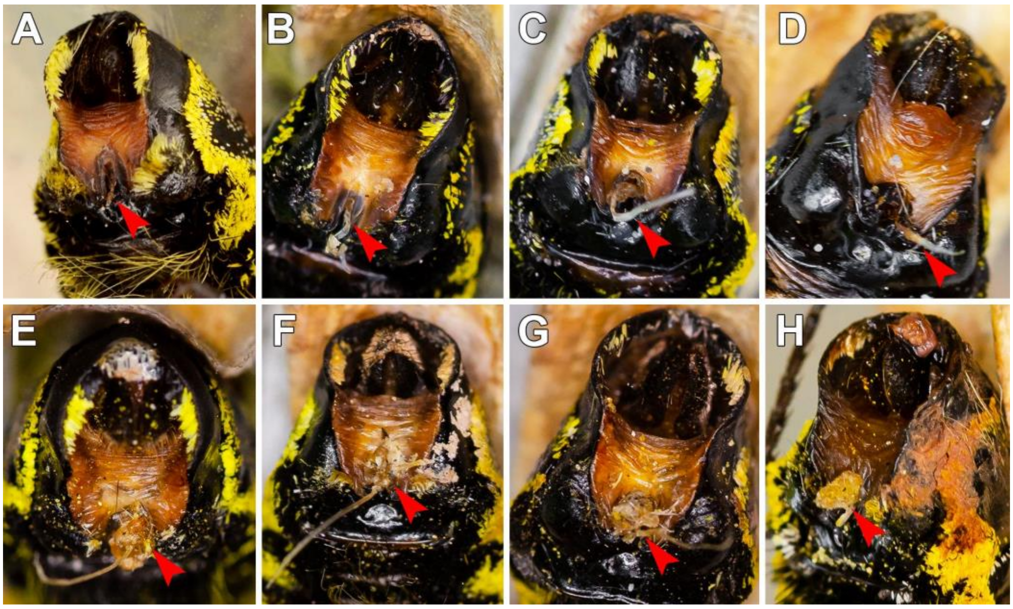


Figure 21. Filament (A–D) and stopple (E–H) diversity in Clouded Apollos. Note the rarely observed very short filament in (A) compared with the common form (C–D). Posterior (E), posterior-ventral (B–D, F–G) and right-ventral views (A, H). The red arrows show the filament or the stopple. Each photograph was taken on different females. Photos were taken by JK.

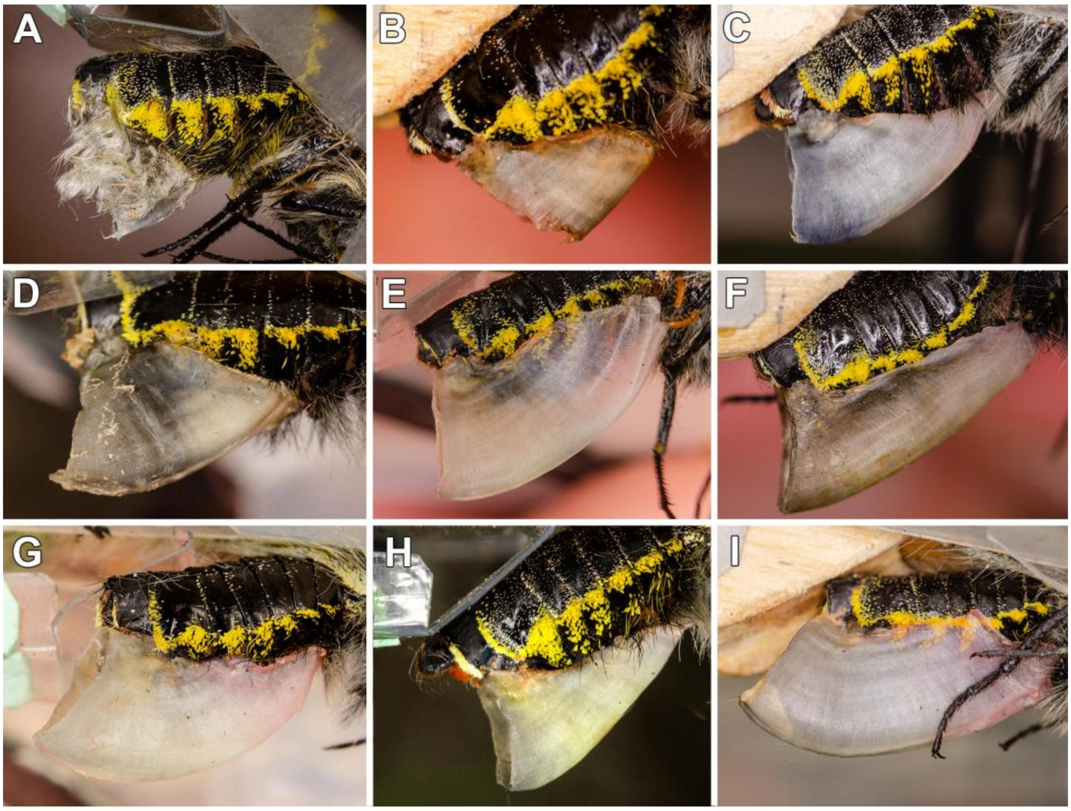
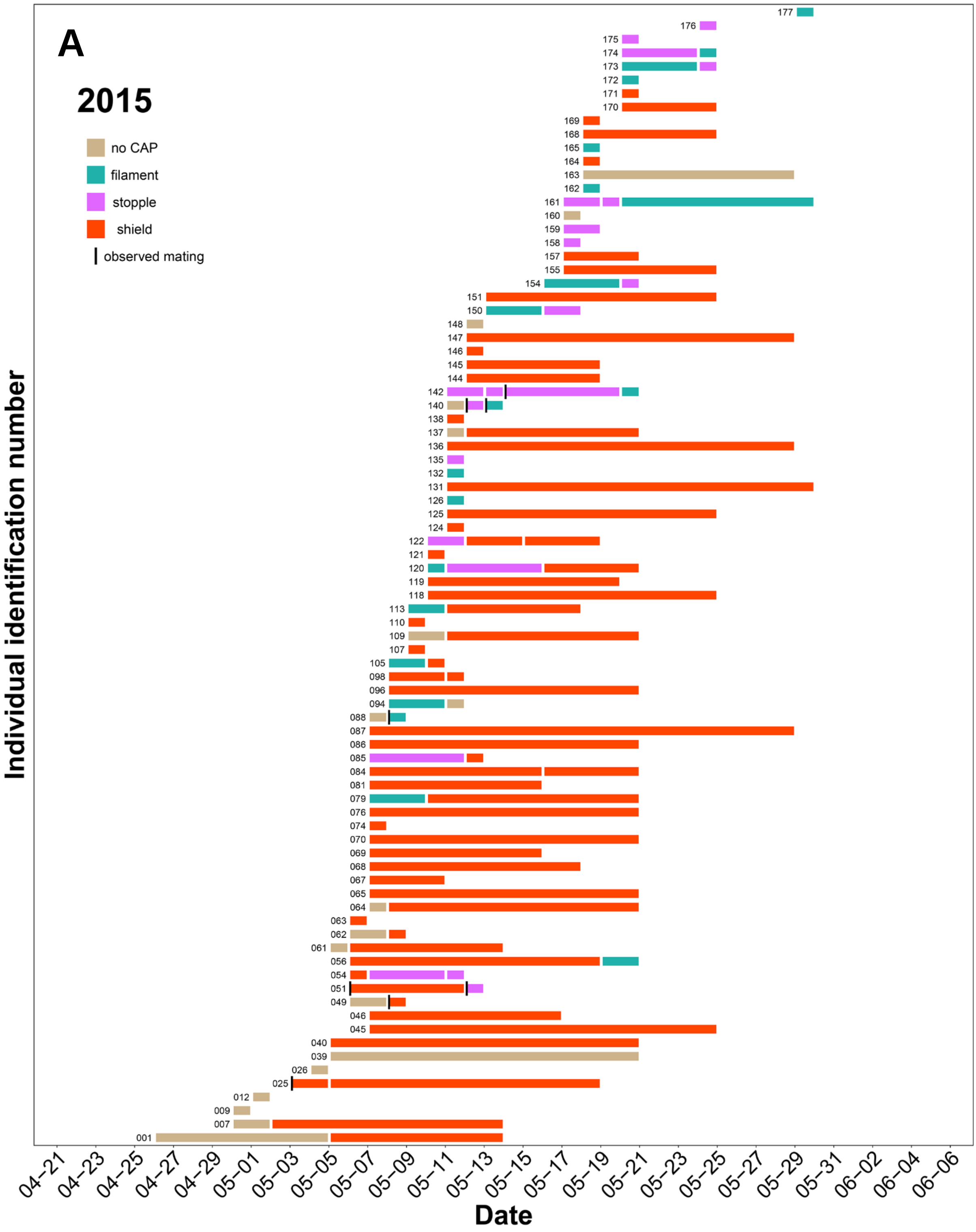


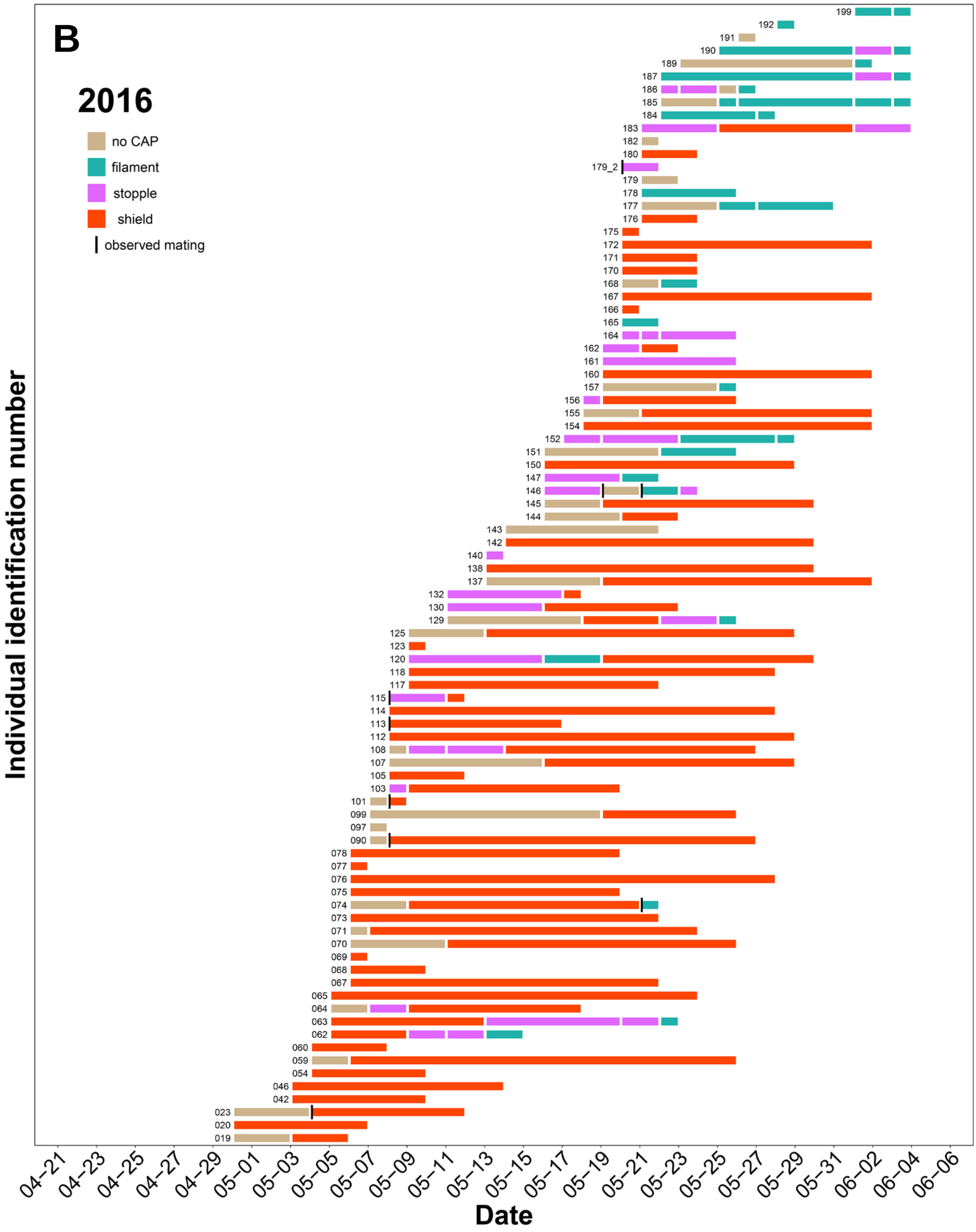
Figure 22. Shield diversity in Clouded Apollos. Note the very short shields rarely observed (A–B); especially (A) could be interpreted as ‘incomplete’. Most shields cover a large part of the females’ ventral abdomen (C–I), and in some cases may nearly reach the thorax, as seen in a rare, extremely long shield (I). Photos were taken by JK.

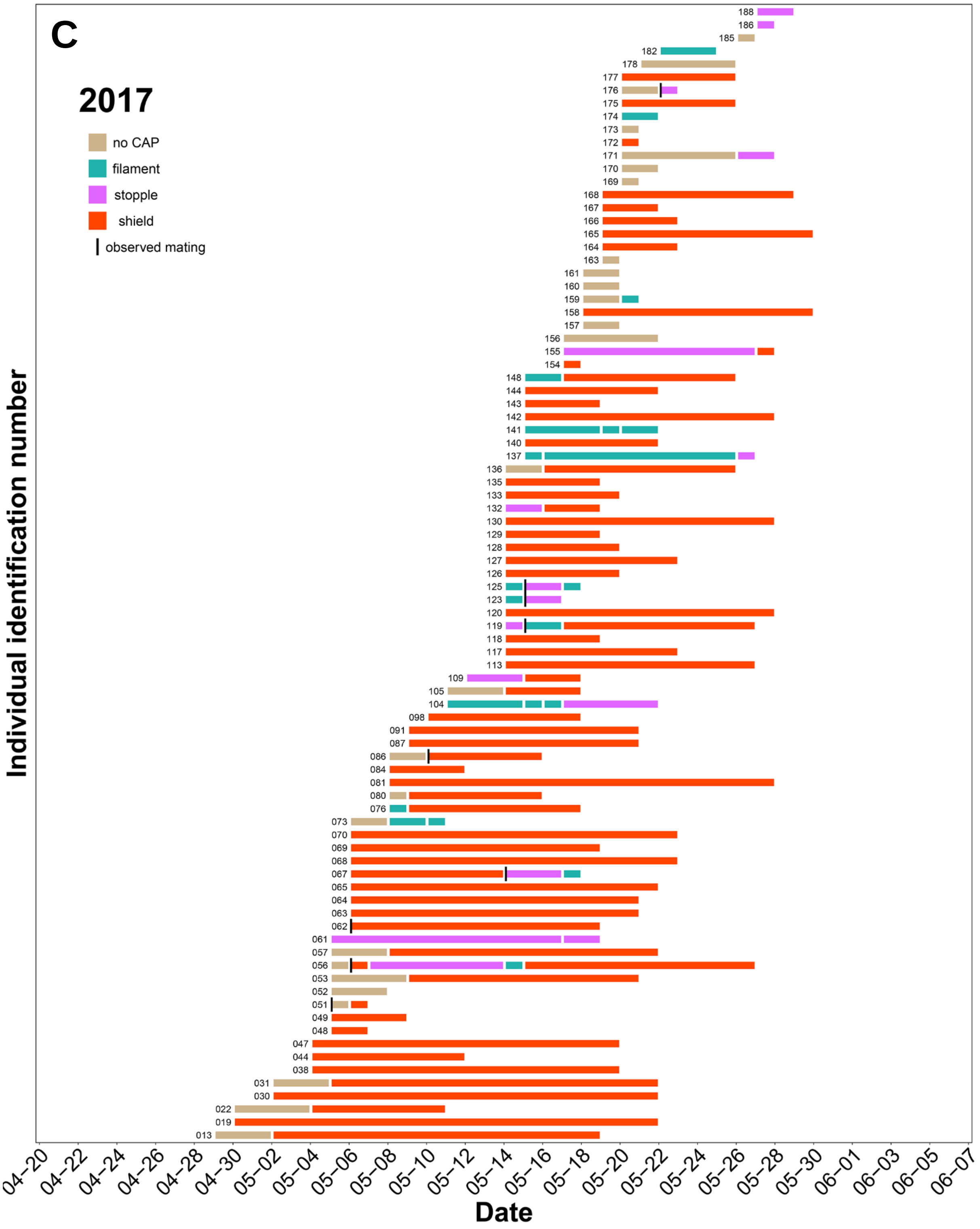
4.2. The dynamics of the mate-guarding devices in Clouded Apollo butterflies

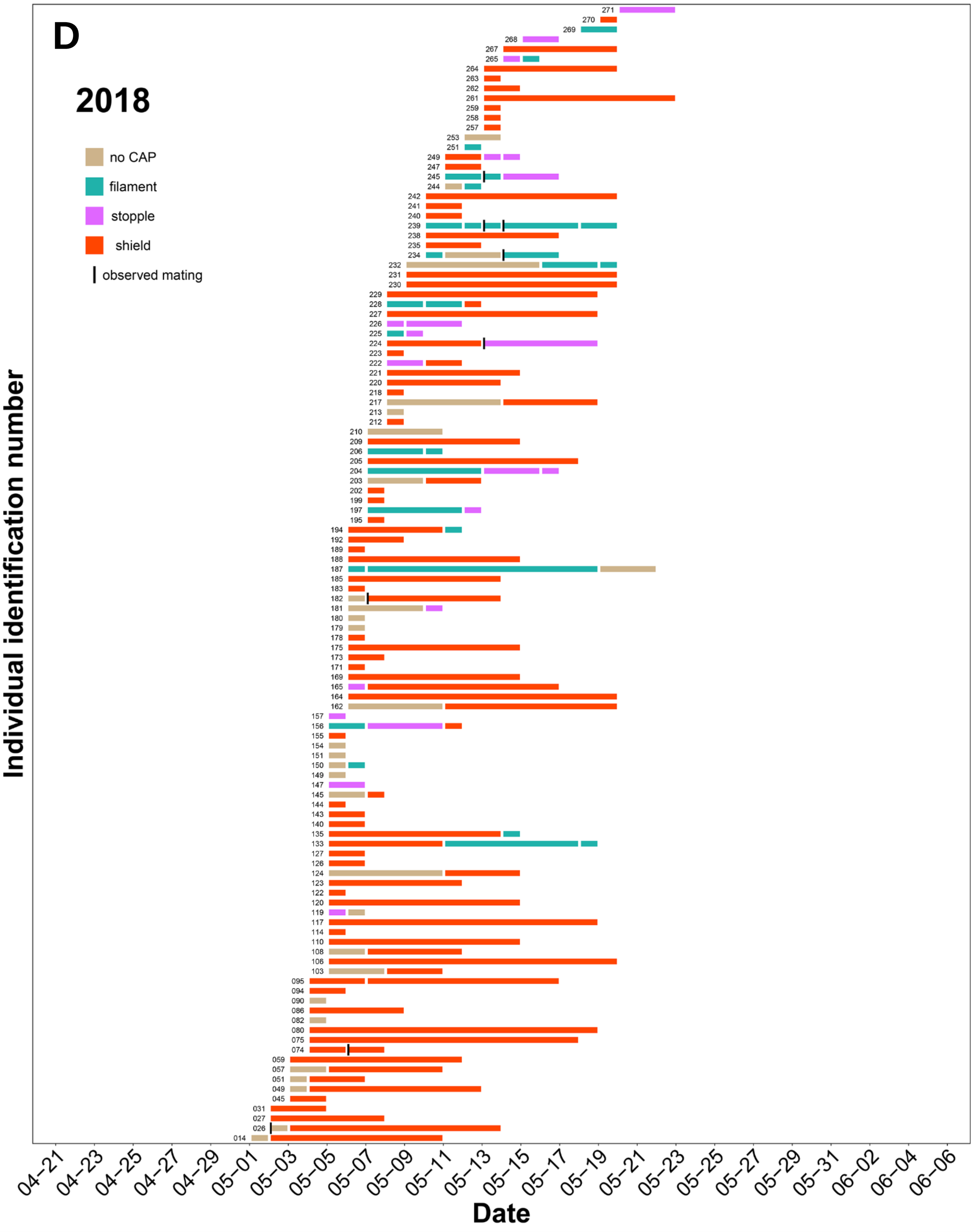
Published as: Gór, Á., Lang, Z., Pásztor, K., Szigeti, V., Vajna, F., & Kis, J. (2023). Mate-guarding success depends on male investment in a butterfly. Ecology and Evolution, 13, e10533. https://doi.org/10.1002/ece3.10533

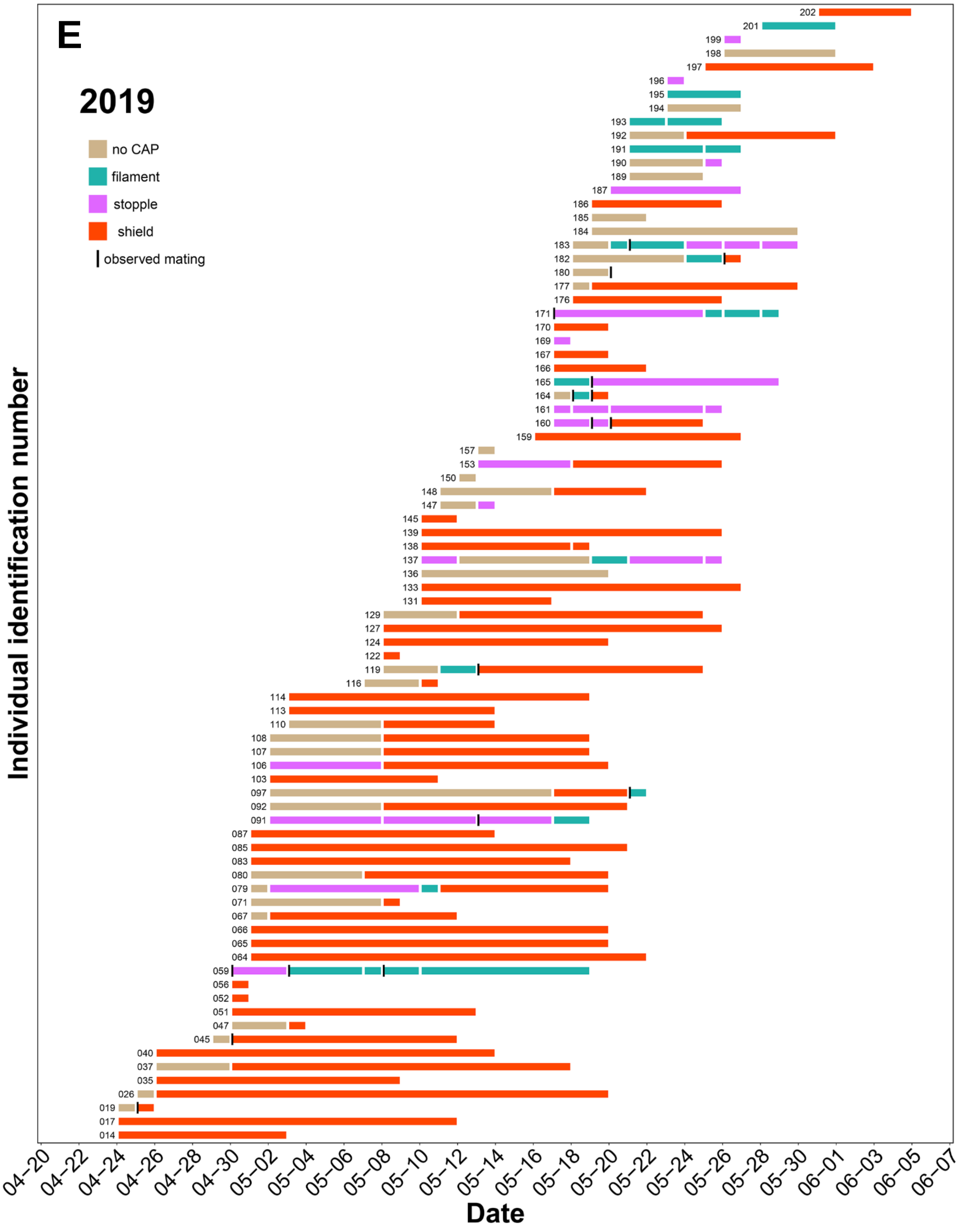
Although we found considerable annual variation in CAP-type event histories, probably due to the variable phenologies, a general, annual pattern emerges (**Figure 23**). Females start their life with no CAP, however we usually find them already mated. At the beginning of the flight period, shielded females are more abundant than later, when the occurrence of small CAPs becomes predominant (**Figure 23**). Furthermore, these figures imply that small CAPs were lost much more frequently and lasted for a shorter period than shields (for further evaluation see 4.2.2. CAP-persistence section).


A
2015
no CAP
filament
stopple
shield
observed mating
Individual identification number
Date
04–21
04–23
04–25
04–27
04–29
05–01
05–03
05–05
05–07
05–09
05–11
05–13
05–15
05–17
05–19
05–21
05–23
05–25
05–27
05–29
05–31
06–02
06–04
06–06
177
176
175
174
173
172
171
170
169
168
165
164
163
162
161
160
159
158
157
155
154
151
150
148
147
146
145
144
142
140
138
137
136
135
132
131
126
125
124
122
121
120
119
118
113
110
109
107
105
098
096
094
088
087
086
085
084
081
079
076
074
070
069
068
067
065
064
063
062
061
056
054
051
049
046
045
040
039
026
025
012
009
007
001


B
2016
no CAP
filament
stopple
shield
observed mating
Individual identification number
199
192
191
190
189
187
186
185
184
183
182
180
179_2
179
178
177
176
175
172
171
170
168
167
166
165
164
162
161
160
157
156
155
154
152
151
150
147
146
145
144
143
142
140
138
137
132
130
129
125
123
120
118
117
115
114
113
112
108
107
105
103
101
099
097
090
078
077
076
075
074
073
071
070
069
068
067
065
064
063
062
060
059
054
046
042
023
020
019
04−21
04−23
04−25
04−27
04−29
05−01
05−03
05−05
05−07
05−09
05−11
05−13
05−15
05−17
05−19
05−21
05−23
05−25
05−27
05−29
05−31
06−02
06−04
06−06
Date


C
2017
no CAP
filament
stopple
shield
observed mating
Individual identification number
188
186
185
182
178
177
176
175
174
173
172
171
170
169
168
167
166
165
164
163
161
160
159
158
157
156
155
154
148
144
143
142
141
140
137
136
135
133
132
130
129
128
127
126
125
123
120
119
118
117
113
109
105
104
098
091
087
086
084
081
080
076
073
070
069
068
067
065
064
063
062
061
057
056
053
052
051
049
048
047
044
038
031
030
022
019
013
04−20
04−22
04−24
04−26
04−28
04−30
05−02
05−04
05−06
05−08
05−10
05−12
05−14
05−16
05−18
05−20
05−22
05−24
05−26
05−28
05−30
06−01
06−03
06−05
06−07
Date


D
2018
no CAP
filament
stopple
shield
observed mating
Individual identification number
Date
04–21
04–23
04–25
04–27
04–29
05–01
05–03
05–05
05–07
05–09
05–11
05–13
05–15
05–17
05–19
05–21
05–23
05–25
05–27
05–29
05–31
06–02
06–04
06–06
271
270
269
268
267
265
264
263
262
261
259
258
257
253
251
249
247
245
244
242
241
240
239
238
235
234
232
231
230
229
228
227
226
225
224
223
222
221
220
218
217
213
212
210
209
206
205
204
203
202
199
197
195
194
192
189
188
187
185
183
182
181
180
179
178
175
173
171
169
165
164
162
157
156
155
154
151
150
149
147
145
144
143
140
135
133
127
126
124
123
122
120
119
117
114
110
108
106
103
095
094
090
086
082
080
075
074
059
057
051
049
045
031
027
026
014


E
2019
no CAP
filament
stopple
shield
observed mating
Individual identification number
202
201
199
198
197
196
195
194
193
192
191
190
189
187
186
185
184
183
182
180
177
176
171
170
169
167
166
165
164
161
160
159
157
153
150
148
147
145
139
138
137
136
133
131
129
127
124
122
119
116
114
113
110
108
107
106
103
097
092
091
087
085
083
080
079
071
067
066
065
064
059
056
052
051
047
045
040
037
035
026
019
017
014
04−20
04−22
04−24
04−26
04−28
04−30
05−02
05−04
05−06
05−08
05−10
05−12
05−14
05−16
05−18
05−20
05−22
05−24
05−26
05−28
05−30
06−01
06−03
06−05
06−07
Date

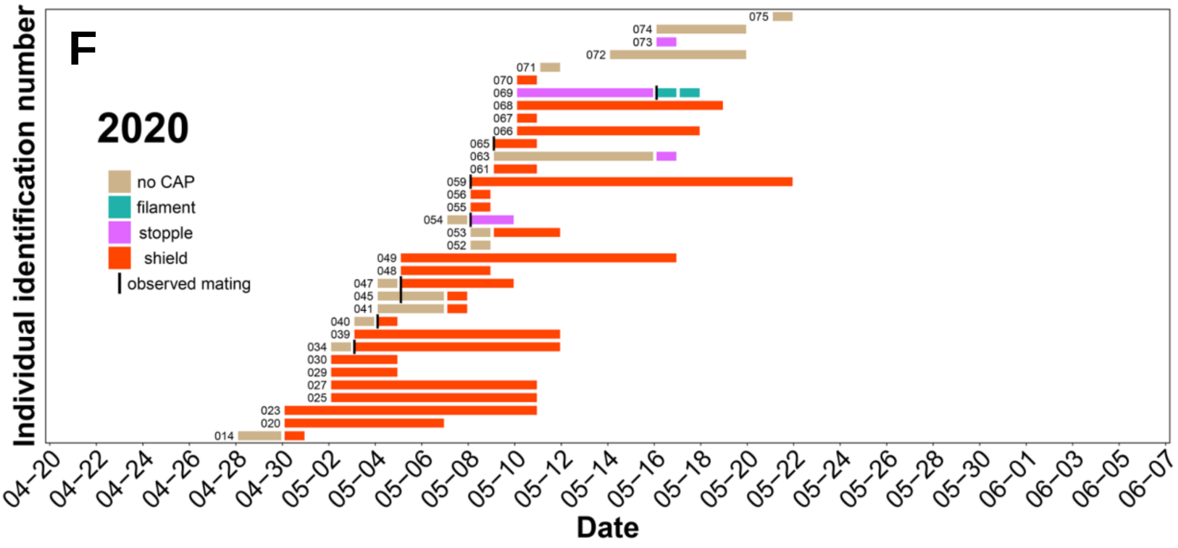


Figure 23. Copulatory opening APpendix (CAP) event histories between 2015 and 2020 (A–F); actual observations on Clouded Apollo females. Each horizontal row (ordered by the annual individual identification numbers from bottom to top) represents the history of a female butterfly and row length shows the individuals' observation period. Colour-coded segments indicate the different CAP-types. Vertical black bars show the observed matings.

Based on the fitted semiparametric multistate survival model, we plotted the proportions of female states according to the time elapsed since the first observations of the individuals (**Figure 24**). Females start their life as no CAP (observed: 150, modelled: 492 females), the proportion of which decreased very steeply in the first few days. During the life of females, there was always a small proportion of those in the state of no CAP or small CAP, and a larger proportion bearing shields. The proportion of individuals bearing a shield decreased more strongly in the last days of the individuals' life, while the proportion of females with small CAP decreased less steeply (**Figure 24**).

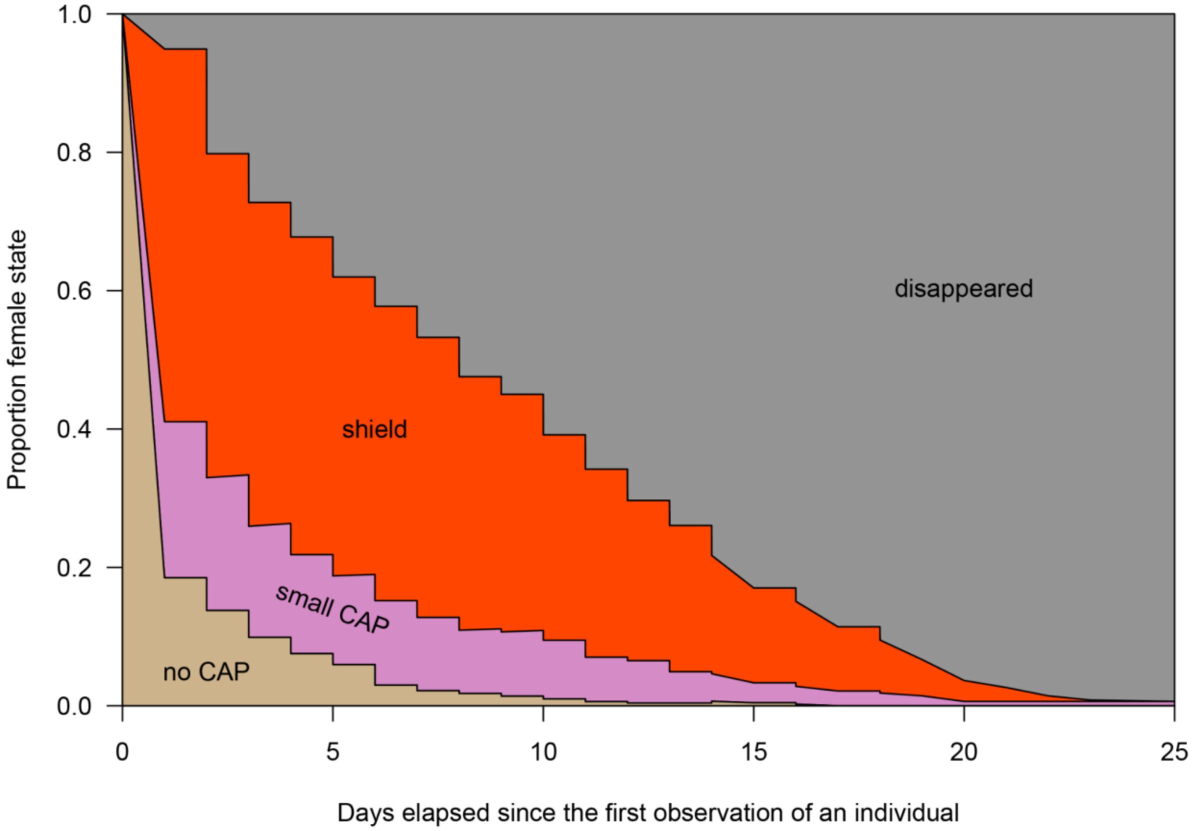


Figure 24. The proportion of each female state over the Clouded Apollo females' life, with the years pooled, based on the fitted semiparametric multistate survival model. Although most females were first caught already bearing a Copulatory opening APpendix (CAP), we assumed that all started their lives in the no CAP state.

4.2.1. CAP-transitions

We observed nearly all possible transitions between states, except the transition from shield to no CAP (**Figure 25**), a transition observed once in this population out of this study's period in 2014.

The numbers of each transition varied across years (**Table 6**). In every year, the most frequent transitions were females with no CAP receiving a shield (315 cases), and shielded females disappearing from the population (333 cases). Scarce transitions were when a small CAP was followed by a no CAP state (7 cases), and when a shield was replaced with a small CAP (16 cases) or another shield (7 cases) (**Table 6**). There were cases when transitions of the same type happened several times in the same female, i.e. (i) a no CAP state followed by a small CAP occurred twice in 4 females, and (ii) a small CAP replaced with another small CAP occurred 2–4 times in 10 females (**Table 6**).

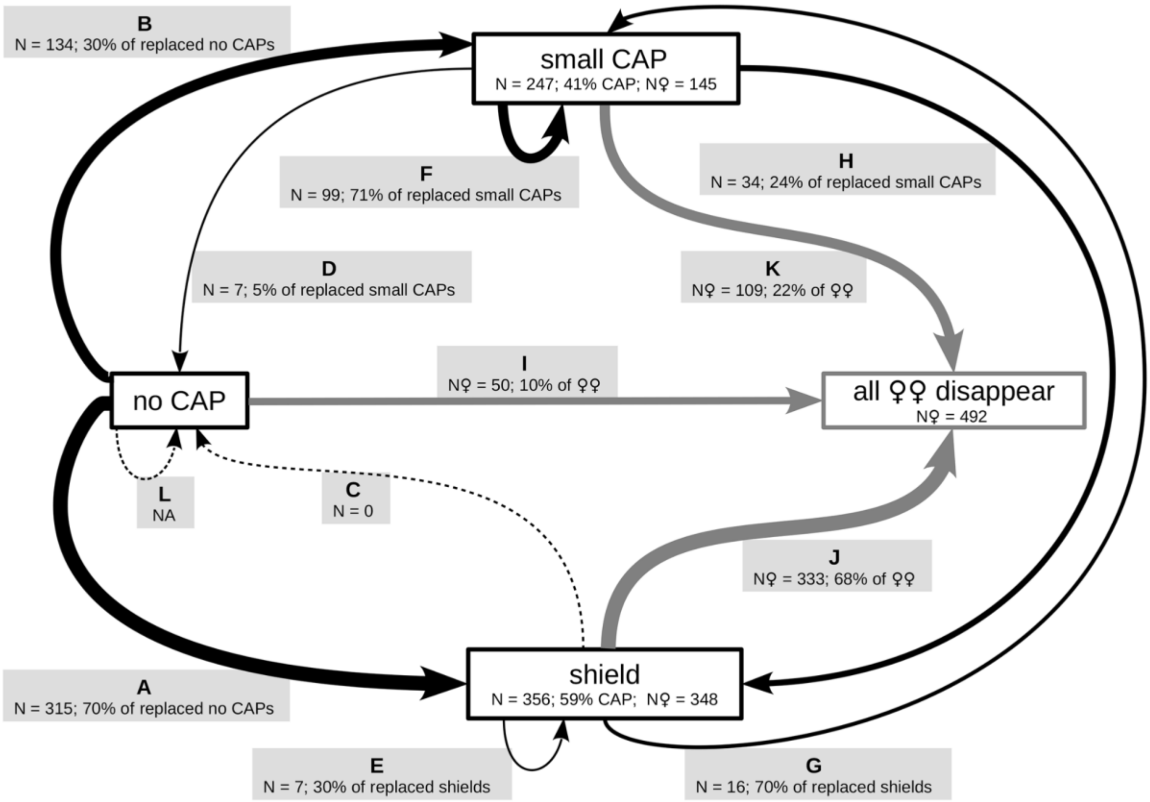


Figure 25. Observed female state transitions (arrows); transitions among Copulatory opening APpendices (CAP) (black arrows, A–H) and female disappearance from the population (grey arrows, I–K) in Clouded Apollo butterflies, 2015–2020. Framed boxes denote female states. The number of states of the entire sample, the percentage of the state relative to all CAPs and the number of females observed with the respective states are shown. We did not provide statistics for no CAPs, since all females were assumed to start their life in this state. The box with all females disappeared (grey frame) shows our entire sample. The boxes attached to black arrows (A–H) show the observed transitions between female states with respective sample sizes and the percentage of transitions within the state the arrow originates from. We did not observe shield loss without replacement (C, dashed line), only prior to this study. Transitions from no CAP to no CAP could not be investigated (L; not available: NA). The boxes attached to grey arrows (I–K) show the number of females and the percentage of their last observed state among all females. Line width of arrows is proportional to the total number of transitions observed, except C and L (width = $\ln(\%_{\text{total transitions}} + 1)$; exact formula was selected upon best visual presentation). Designed by JK.

Table 6. Frequencies of the different transitions over six years in Clouded Apollo females, used in the semiparametric multistate survival model. The table shows the annual and the total number of transitions with the number of females gone through a specific transition in parentheses. Although most females were first caught already bearing a CAP, we assumed that all started their lives as no CAP. Transitions to disappeared show the last observed state of any female, thus all these states and females overlap with those of other transitions, since ultimately, all females disappeared by the end of the flight period.

Year / Transition	2015	2016	2017	2018	2019	2020	Total	Ref to Fig. 25.
no CAP → small CAP	26 (26)	33 (31)	20 (20)	26 (25)	25 (24)	4 (4)	134 (130)	B
no CAP → shield	51 (51)	52 (52)	56 (56)	81 (81)	50 (50)	25 (25)	315 (315)	A
no CAP → disappeared	8 (8)	5 (5)	11 (11)	12 (12)	9 (9)	5 (5)	50 (50)	I
small CAP → no CAP	1 (1)	2 (2)	0	3 (3)	1 (1)	0	7 (7)	D
small CAP → small CAP	12 (9)	25 (16)	15 (10)	19 (14)	24 (11)	4 (1)	99 (61)	F
small CAP → shield	6 (6)	10 (10)	7 (7)	4 (4)	7 (7)	0	34 (34)	H
small CAP → disappeared	22 (22)	26 (26)	15 (15)	24 (24)	18 (18)	4 (4)	109 (109)	K
shield → small CAP	3 (3)	5 (5)	2 (2)	5 (5)	1 (1)	0	16 (16)	G
shield → shield	4 (4)	0	0	2 (2)	1 (1)	0	7 (7)	E
shield → disappeared	54 (54)	57 (57)	61 (61)	80 (80)	56 (56)	25 (25)	333 (333)	J
Total	187 (84)	215 (88)	187 (87)	256 (116)	192 (83)	65 (34)	1102 (492)	

4.2.2. CAP-persistence

The average risk over time to lose a small CAP (solid red and green lines; **Figure 26**) was significantly higher than the average risk over time of losing a shield (dotted blue and magenta lines; **Figure 26**) during the entire range of the individuals' observation period, as indicated by the lack of overlap between confidence regions (**Figure 26**). The transition from small CAP to no CAP (solid black line; **Figure 26**) is a poor estimate (see wide grey confidence region) and did not significantly differ from losing a shield (**Figure 26**).

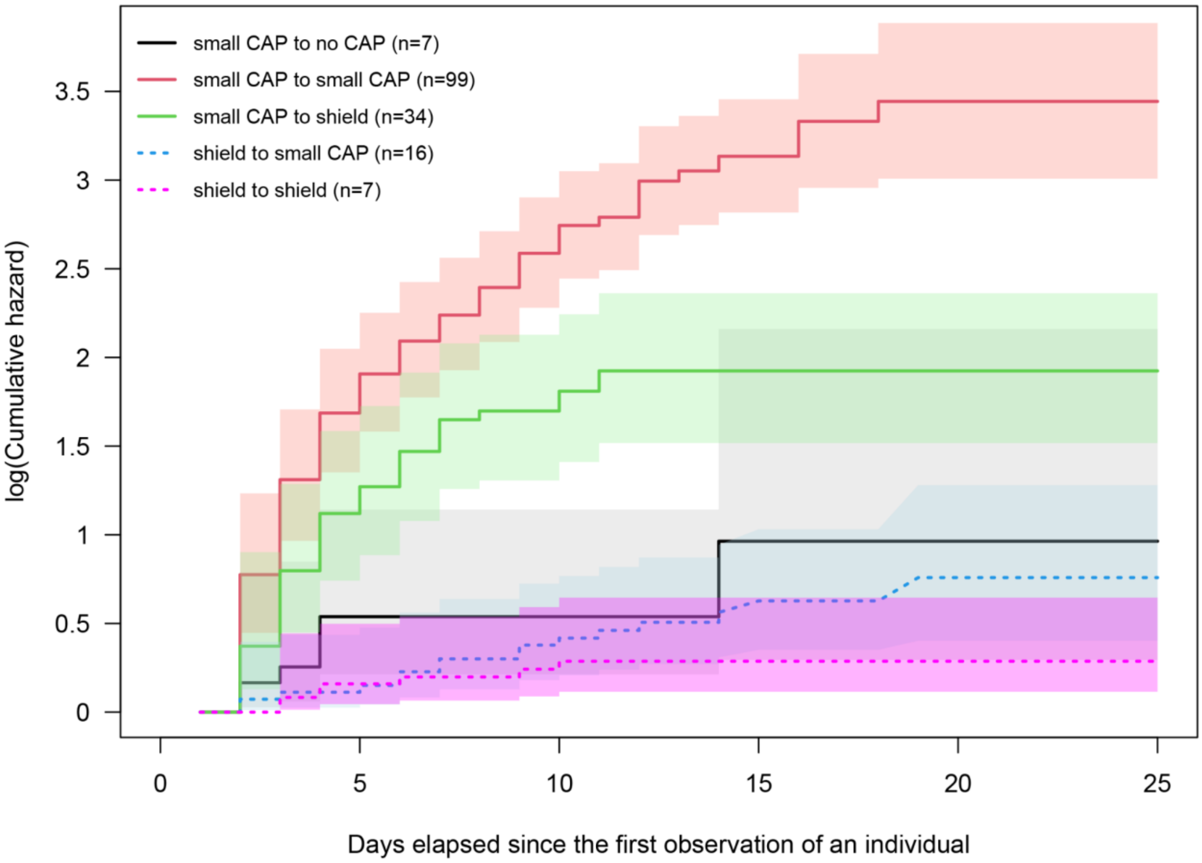


Figure 26. Cumulative hazards of small CAP (Copulatory opening APpendix) and shield losses in Clouded Apollo females from the semiparametric multistate survival model at a given observation time. Lines show the hazard functions, shadings are the 99.16% confidence intervals of the functions (Bonferroni-corrected CI-s for six comparisons: three transitions from small CAP were compared to the shield to small CAP (3 comparisons) and to the shield to shield transitions (3 comparisons)).

4.2.3. Proportional hazard submodels

In transitions starting from the no CAP state ($n_{\text{females}} = 492$; $n_{\text{transitions}} = 499$), the risk of transitioning to a shield (relative risk; RR = 2.38; $p < 0.001$; CI: 1.95, 2.92) was 2.38× larger, and the risk of disappearance at a given time point (RR = 0.36; $p < 0.001$; CI: 0.26, 0.51) was 0.36× smaller than transitioning to a small CAP. Time interactions had no significant effects ($p = 0.988$ and $p = 0.127$).

In transitions starting from a small CAP ($n_{\text{females}} = 145$; $n_{\text{transitions}} = 247$), the risk of transitioning to another small CAP (RR = 2.98; $p < 0.001$; CI: 1.86, 4.77) was at least 2.98× larger, and the risk of disappearing at a given time point (RR = 1.77; $p = 0.018$; CI: 1.11, 2.85) was at least 1.77× larger than transitioning to a shield. The time interaction was significant for the transition from small CAP to small CAP (RR = 1.08; $p = 0.049$; CI: 1.00, 1.17) and for the transition from small CAP to disappearance (RR = 1.16; $p < 0.001$; CI: 1.08, 1.25) which resulted an increase in the relative risks over time.

In transitions starting from a shield ($n_{females} = 348$; $n_{transitions} = 356$), the risk of disappearance at given time point (RR = 21.43; $p < 0.001$; CI: 12.84, 35.76) was 21.43× larger than transitioning to a small CAP, meaning that shields usually persist until the end of an individual's life. There was no significant difference in risk between transitioning from a shield to a small CAP and to another shield (RR = 0.82; $p = 0.763$; CI: 0.22, 3.01). Time interactions were not significant ($p = 0.581$ and $p = 0.447$).

4.2.4. Models with covariates

To decide whether a shield is long or short, we plotted the lost (red triangles) and permanent shields' (black dots) length and the 90th percentile of lost shields (green line). Shields below this value were short, above it, long (**Figure 27**).

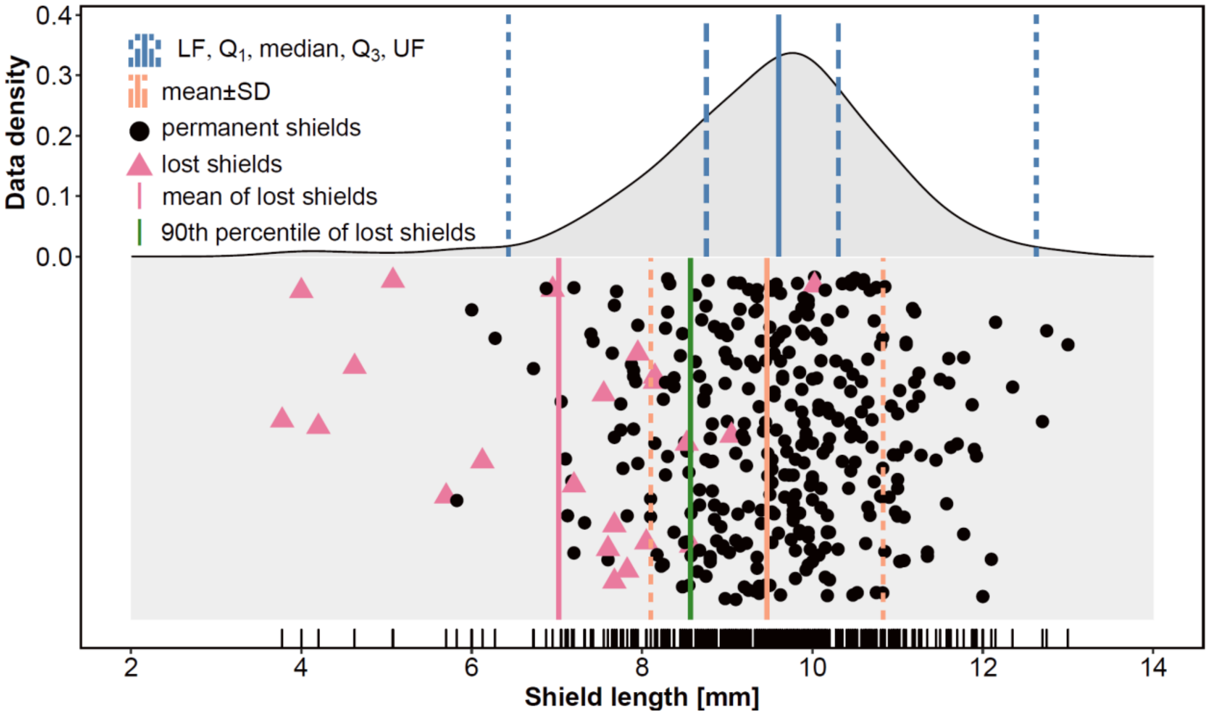


Figure 27. The distribution of shield lengths for the six years combined in Clouded Apollo females. Note the difference between the lost (red triangle) and the permanent (black dot) shields. Blue vertical lines show the lower fence (dashed; LF), first quartile (longdashed; Q_1), median (solid), third quartile (longdashed; Q_2) and the upper fence (dashed; UF) for all shields. Orange lines represent the mean (solid) and standard deviation (dashed; SD) of permanent shields. Lower fence = $Q_1 - (1.5 \times \text{IQR})$, upper fence = $Q_3 + (1.5 \times \text{IQR})$. The vertical red line shows the mean of lost shields and the vertical green line indicates the 90th percentile of lost shields. Above this value, shields were long, below shields were short. Data points are jittered along the y-axis for better visibility. We observed a total of 23 shields lost, but only 22 of those were measured.

According to the submodel on the relationship between shield loss and shield size ($n_{females} = 348$; $n_{transitions} = 1205$), the risk of transitioning from a shield to a small CAP for short shields was 27.56× larger than for the long shields (RR = 27.56; $p < 0.001$; CI: 5.83, 130.40). In addition, the risk of transitioning from a shield to another shield for short shields was 25.42× larger than for long shields (RR = 25.42; $p = 0.003$; CI: 2.96, 218.60). In summary, shorter shields were more prone to be lost than longer ones.

According to the submodel on the relationship between transitions and female sizes ($n_{\text{females}} = 492$; $n_{\text{transitions}} = 1190$), the risk of receiving a shield either after a no CAP state (RR = 3.70; $p = 0.012$; CI: 1.34, 10.23) or a small CAP (RR = 503.70; $p = 0.002$; CI: 9.06, 2.80×10^{4}) was significantly higher in females with wider thoraces than in females with narrower thoraces; i.e. females with wider thoraces are more prone to receive shields than narrower females. Moreover, the risk of disappearance at a given time point either after bearing a small CAP (RR = 7.16×10^{-4}; $p < 0.001$; CI: 7.56×10^{-5}, 6.79×10^{-3}) or a shield (RR = 0.05; $p < 0.001$; CI: 0.01, 0.18) was significantly lower in females with wider thoraces than in females with narrower thoraces.

Similarly, the risk of receiving a shield after a no CAP state was significantly higher in females with wider heads than in females with narrower heads (RR = 3.99; $p < 0.001$; CI: 1.89, 8.42). However, no significant relationship was found between the risk of receiving a shield after a small CAP and female head width (RR = 16.75; $p = 0.133$; CI: 0.42, 662.50).

Moreover, the risk of disappearance at a given time point either after a no CAP state (RR = 9.68×10^{-3}; $p < 0.001$; CI: 6.56×10^{-4}, 1.43×10^{-1}) or after bearing a small CAP (RR = 0.15; $p < 0.037$; CI: 0.02, 0.89) or a shield (RR = 0.35; $p = 0.026$; CI: 0.14, 0.88) was significantly lower in females with wider heads than in females with narrower heads.

Female body mass, wing and proboscis length were not significantly related to transitions in female state or were non-interpretable (see **Appendix 1**). Taken together, both females with wider thoraces, when controlled for head width, and females with wider heads were more prone to receive shields and less prone to disappear at a given time point than females with narrow thoraces, while other measures of body size had no significant effects.

The submodel on the relationship between transitions and ASR ($n_{\text{transitions}} = 1206$) showed that the risk of receiving a shield either after a no CAP state (RR = 3.67; $p < 0.001$; CI: 2.21, 6.09) or a small CAP (RR = 7.04; $p = 0.001$; CI: 2.12, 23.41) was higher when the population was male-biased.

Furthermore, the risk of disappearance at a given time point either after a no CAP state (RR = 0.38; $p = 0.003$; CI: 0.21, 0.72) or after bearing a small CAP (RR = 0.31; $p < 0.001$; CI: 0.20, 0.47) or a shield (RR = 0.41; $p < 0.001$; CI: 0.29, 0.58) was lower when the population was male-biased. In addition, the risk of receiving a small CAP after a no CAP state (RR = 0.49; $p < 0.001$; CI: 0.33, 0.72) was also lower when the population was male-biased.

4.2.5. Time series analysis

Both the proportion of shields and the proportion of males in the population decreased with time (**Figure 28**).

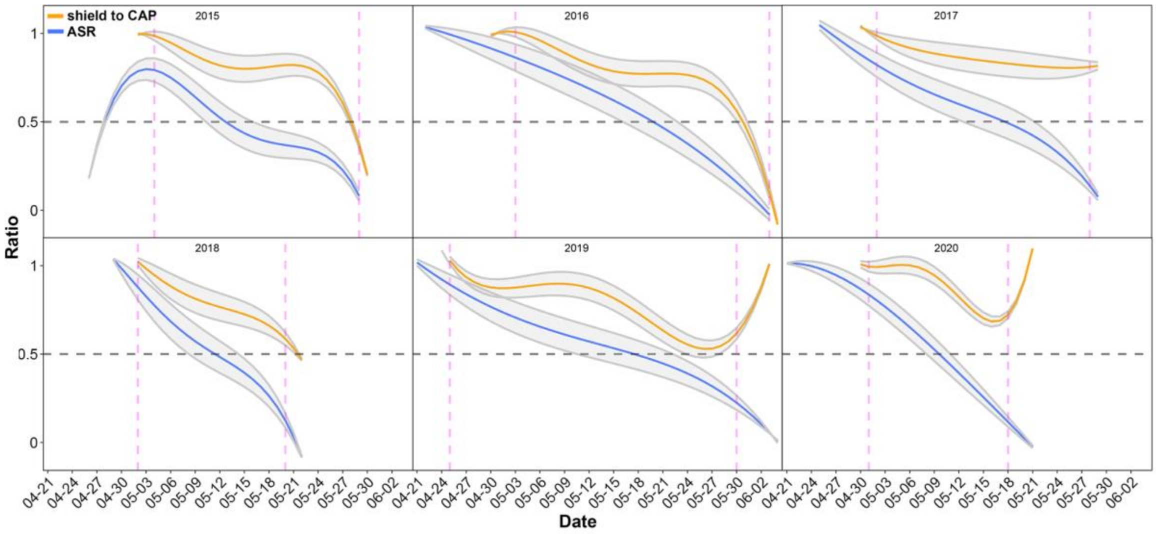


Figure 28. Shield-to-CAP (Copulatory opening APpendix) ratio (orange curves) and adult sex ratio (ASR, blue curves) changes over the flight periods in six years in Clouded Apollos. Grey shading width is proportional to the number of individuals (width = ln(daily n_{ind} / 50); exact formula was selected upon best visual presentation). The areas between the vertical dashed lines are intervals with at least 5 females observed in the population each day. We think that out of these intervals, ratio estimates could be severely biased. The horizontal dashed lines show equal numbers of shields and small CAPs or males and females for a given day; at 0 only small CAPs or females, at 1 only shields or males were present in the population.

In line with **Figure 28**, the change in shield-to-CAP ratio was significantly related to the change in ASR with a three-day lag ($n_{observations}$ = 118; elasticity = 0.19; *p* = 0.003; CI: 0.07, 0.32; *GLMM*). Here, elasticity is interpreted as a 1% decrease in daily ASR involves, on average, 0.19% decrease in daily shield-to-CAP ratio. Year as a random factor explained only an extremely small proportion of the total variance (0.89%).

5. Discussion

We identified three different mate-guarding devices in the 492 Clouded Apollo females surveyed, with some observed without devices for a period of time. Different types could be identified on the same females consecutively in some cases. At least some of the device losses can be attributed to removal by males. We found large variation both in shield dimensions and the shapes of all CAP-types.

Furthermore, we found that (i) shields were less prone to be lost than small CAPs and (ii) longer shields were less prone to be lost than shorter ones. (iii) Females with wider thoraces were more prone to receive shields than narrower females and (iv) females with narrower thoraces had a higher risk to disappear at a given time point than wider ones. (v) Females were more prone to receive a shield when the population was male-biased and receive a small CAP or disappear when the population was female-biased. (vi) ASR and shield-to-CAP ratio decreased with time during the flight periods and the decrease in ASR was followed by the decrease of the shield-to-CAP ratio three days later.

5.1. Terminology

We introduced the term Copulatory opening APpendix (CAP) and its three types, filament, stopple and shield. These categories are applicable for (i) non-invasive, external scrutiny, and allow surveying natural populations, (ii) they help to understand the within-population variation in males' investment in mate-guarding devices at a specific mating attempt in the Clouded Apollo butterfly, (iii) emphasise the structural relationships among these devices and (iv) avoid ambiguity with the general terms, used mostly to distinguish species-specific devices found in a wide range of taxa. For example, the terms 'mating plug' and 'copulatory plug' are used synonymously for devices to impede male intromission and found in a wide range of taxa, from nematodes (Timmermeyer et al. 2010) to primates (Danzy et al. 2009, Dixson & Anderson 2002, Dunham & Rudolf 2009) and maybe applied to internal plugs (e.g. Lepidoptera: Papilionini; Matsumoto & Suzuki 1995) as well as plugs that also externally block the copulatory opening (Canales-Lazcano et al. 2019). The term 'sphragis' is defined as 'an external formation, originating from male accessory glands, with a well-defined, species-specific structure, fixed to the female abdomen following insemination, where it blocks the ostium bursae' (Carvalho et al. 2017), thus denoting various structures. In the case of Clouded Apollos, we emphasise that all three CAP-types showed considerable size variation as well as some variation of form. The stopple overlaps with the notion of the mating plug, but larger stopples may rather correspond to the term 'vestigial sphragis' (category 1, sensu Carvalho et al. 2017). The shield covers the term of the hollow type of large sphragis (category 4a, sensu Carvalho et al. 2017; see also Orr 1995) and, in contrast to stopples and filaments, has been well-known in Clouded Apollos (Orr 1988, 1995, Vlašánek & Konvička 2009). Orr's (1995) assumption that in papilionid butterflies the sphragis is derived from the spermatophore rather than an enlarged mating plug and our emphasis on the continuity from filament to stopple and from stopple to shield further justifies a distinct terminology. Currently, this terminology is valid only to Clouded Apollos, although it could probably be extended to at least some *Parnassius* species, especially in the *Driopa* subgenus of *Parnassius*, which the Clouded Apollo belongs to (Condamine et al. 2018, Zheng et al. 2018), although this requires detailed studies of those species. Note that we found all three CAP-types in several different populations in Hungary, some in the same region as this study, others from distant habitats, some in several years (**Table 7**). These indicate that the three CAP-types are likely regular traits of Clouded Apollo populations.

Table 7. Habitats where we conducted observations on Clouded Apollo butterflies. We found all three CAP-types, filaments, stopples and shields in all locations. Region is the larger geographical area, location is the locality of a habitat within a region. Distance and direction are relative to the present study site. Within Visegrádi-hegység, habitats are separated by dense oak and beech forests. Among regions, vast areas unsuitable for Clouded Apollos are situated. For reference, the first line shows data from the study site detailed in this thesis.

Region, location	Longitude, latitude	Altitude (m, a.s.l.)	Distance (km)	Direction	Years
Visegrádi-hegység, Hegyesd (present study)	47.756411, 19.047897	295			2013 – 2022
Visegrádi-hegység, Leánykúti-rét	47.739948, 19.059378	297	2.0	southeast	2008 – 2013, 2021
Visegrádi-hegység, Teknős-hát	47.743184, 19.033955	569	2.5	southwest	2021
Visegrádi-hegység, Vízverés nyerge	47.757435, 19.009285	495	2.9	west	2021
Visegrádi-hegység, Pap-hegy	47.780686, 19.007177	410	4.0	northwest	2021
Visegrádi-hegység, Kőrös-hegy	47.787422, 19.011416	353	4.4	northwest	2021
Visegrádi-hegység, Sóstói-rét	47.775703, 19.004207	423	3.9	northwest	2021
Visegrádi-hegység, Nagy-erdő	47.776591, 18.994735	362	4.6	northwest	2021
Börzsöny, Nagy-Hideg-hegy	47.936432, 18.922600	852	22.1	northwest	2008 – 2022
Budai-hegység, Iharos	47.455500, 18.849834	300	36.6	south	2017 – 2022
Vértes, Fáni-völgy	47.460090, 18.443680	289	56.0	southwest	2019
Aggteleki-karszt, Tohonya-völgy	48.497233, 20.537271	271	137.9	northeast	2017 – 2020

5.2. CAP-material and origins

The three CAP-types look to be made of the same material, although this should be investigated with chemical analysis that we did not perform. Sphragis material in Lepidoptera is supposed to be produced by the male accessory glands (**Figure 10**A; also Figure 4.7a in Orr 1988); *c.f.*

Parnassius apollo, Figure 1 in Eltringham 1925). Shield material is released from around the valvae. We have never observed this when only stopples or filaments were produced. This implies a multi-channel system that conducts stopple material through the aedeagus and shield material through a lot of thin channels that open along the membrane between the anterior edges of the valvae and the 8th sternite (ventral abdominal segment of the cuticle; **Figure 10**B: magenta arc; see also **Figure 10**C, D, **Figure 14**A–C; Orr 1995). The shield surface's striation along its anterior-posterior curve supports the hypothesis that material is secreted through a series of narrow openings (**Figure 10**I, **Figure 22**I). The pair of valvae may serve as a mould to shape the shield on its external surface. This is supported by the medial suture on the shield (**Figure 10**G, H, **Figure 11**E) that may be formed by the small cleft between the medial edges of the valvae (**Figure 10**B, **Figure 30**A, B, D, E). These assumptions imply that, in contrast to **Figure 10**A, secretion from the accessory glands is not only released via the aedeagus, but also via channels around the valvae (**Figure 10**B–D) that future anatomical studies should test. This seems to be a different male moulding mechanism, compared to the sphragis-building papilionid butterfly *Euryades corethrus* (Lepidoptera: Papilionidae), where the male's sacs within the abdomen moulds parts of the sphragis during copulation (Orr 1988 and Figure 29 in Carvalho et al. 2017). All CAPs in Clouded Apollos are likely attached with an internal plug inserted in the female vagina (ductus bursae; **Figure 10**F; *c.f.* Figure 4.6a in Orr 1988; Figure 16.1a in Orr 1995).

5.3. CAP-formation

Similarity in material, large variation in the size of all CAP-types, as well as the frequent presence of threads similar to the filament on stopples (shielded or not) suggest investment-dependent continuous and non-linear (shields are conspicuously much larger than small CAPs) transitions from filament to stopple and from stopple to shield, created during shorter or longer periods of seemingly the same procedure, even if the outcome is manifested as three morphologically distinct categories of the CAP-types, that are variable in size. We assume that, by males increasing material and time investment in mate-guarding, potential internal plugs in the vagina (**Figure 10**F) may develop further to filaments, stopples, then shields. This is supported by the following arguments: (i) a filament may be produced when the male retracts its aedeagus from the bursa copulatrix with still unhardened CAP-material on its tip. This could explain why threads can be found not only in the copulatory opening, but often also on top of the stopple, even when the stopple is shielded, suggesting that the aedeagus has an important role in forming the stopples (**Figure 12**C, **Figure 21**E–G). A plug may be inserted in the vagina after insemination; (ii) the amorphous stopples may be produced when males are able and willing to invest more in mate-guarding. Since a stopple fills the sinus of the copulatory opening (ostium bursae; **Figure 10**E, F, **Figure 21**E–H), often capping it entirely, this may make it more difficult for rivals to penetrate the vagina, compared to just boring through an internal plug with the aedeagus.

Stopples with double or triple filament-like threads (**Figure 12**A, B) indicate that males may try to penetrate beside or through the stopple and may produce another one (Dickinson & Rutowski 1989, Matsumoto & Suzuki 1995); (iii) the possibility of CAP-removal may have driven the evolution of male investment into mate-guarding further by benefiting males to invest even more resources, if available, in producing a shield around the stopple to prevent stopple removal. We assume that investment into material is proportional to the effectiveness of CAPs preventing the females from future mating, i.e. filaments may simply signal small, internal plugs easy to remove, while stopples may provide better protection and shields mostly last for life.

'Incomplete sphragides' were observed in several butterfly species (Carvalho et al. 2017, 2019, Matsumoto 1987, Orr 1988). In Clouded Apollos, these may be equivalent to the small CAPs, the stopple and the filament that may be easily overlooked in mark-recapture studies not designed to record these types (Clouded Apollos, Czech Republic; Vlašánek & Konvička 2009). We found a few sphragides with very short shields that could be considered 'incomplete' (**Figure 22**A, B). However, we emphasise that a continuous concept of investment helps a better understanding of CAP-variation than a hypothesised aim to produce a 'complete' shield. At any given mating attempt, male capacity, the mates' relative condition and disruptive factors, such as rivals or predators may determine the outcome of CAP-formation.

Capping a female for life is possible due to the separate openings for copulation and oviposition. In fact, all butterflies belong to the group Ditrysia, involving this reproductive architecture (**Figure 10**E, F, I; Scoble 1992). The copulatory opening is surrounded by a large, heavily sclerotized hairless surface, called sterigma (**Figure 10**E, F). The sterigma makes for males more difficult to grab the female, position themselves into the 'mating triangle' and fix the sphragis (Orr 1995). It potentially helps avoiding injuries during mating (Dickinson & Rutowski 1989, Kawagoe et al. 2001, Nilakhe 1977, Orr 1999, Thornhill & Alcock 1983). This surface is less exposed and usually covered with scales in most (non-sphragis bearing) butterflies (Summers et al. 2020). We assume that the aedeagus has an important role in inserting the internal plug, building the stopple and blending stopple and shield material, albeit we could not observe this. The valvae and the aedeagus can flexibly move (**Figure 10**B, C; **Video 4**, 0:37). In copula, the male probably holds the female with its two pairs of unci (**Figure 10**D, I, **Figure 13**A, D), while the valvae are likely play a role in shaping the shield of the sphragis (**Figure 13**B, C, E, F).

5.4. Lack of sphragis and CAP-removal

In natural populations of *Parnassius mnemosyne* (Vlašánek & Konvička 2009), *Parnassius clodius* (Auckland et al. 2004, Calabrese et al. 2008) and *Parnassius smintheus* (Calabrese et al. 2008, Matter et al. 2012), sphragides have not been found in a small proportion of the females, mostly considered unmated, but Vlašánek & Konvička (2009) proposed they could lose the sphragis and we suggest some of the females could bear small CAPs unnoticed by the observers.

During this study, females with no CAP have been recorded as freshly emerged early in the flight period, or had been previously observed with CAPs that were lost later in their lives, late in the flight period. This clearly indicates that the lack of a CAP neither informs on virginity, nor on the presence of an internal plug in the vagina, i.e. if the female has already mated.

There are a few reported examples of sphragis loss (Marshall 1901, Petersen 1928, Matsumoto unpublished data) attributed to removal (Matsumoto & Suzuki 1992, 1995, Vlašánek & Konvička 2009) or spontaneous loss (Pierre 1985, Vlašánek & Konvička 2009). One relatively short-shielded sphragis fell off when we measured a female, suggesting that a few loosely attached sphragides could indeed be lost spontaneously. We observed sphragis removal by males in a few cases, when the male attempts to grasp the shield with its valvae and unci (**Video 4**, 0:16), and may secrete a fluid that softens shield material (Orr 1988). Shield shape may be considerably distorted during this process (**Figure 18**A–P) and this could not happen without softening. In a few cases we found shields torn (**Figure 18**A, B, E, F, I–P), likely without being softened. If the shield wall is thin, it might be torn by the valvae, although we have no direct evidence. After grabbing the shield, the male pulls the sphragis away from the female body to try to loosen it in the vagina (**Figure 10**I; **Video 4**, 0:00), while the aedeagus was observed to thrust towards the female abdomen (**Figure 16**J, L; **Video 4**, 0:37). Since the shield impedes the observation of the sharp-pointed aedeagus, it is difficult to assess its role in removing the shielded stopple. We found perforations on a handful of shields (**Figure 17**A–E, H) and the shape of these circular perforations indicates that they were bored through the shield in a softened condition from the external surface with a tool similar in diameter to the aedeagus. However, these attempts are likely futile, because the shield keeps the aedeagus far from the copulatory opening to exercise enough force to be effectively used as a lever (**Figure 10**G–I, **Figure 16**A–L). However, the role of the valvae looks clear. When the sphragis is loosened enough, sometimes rotated from its original position, the valvae seek to cut the stopple neck and sever the shielded stopple (**Figure 10**H; **Video 4**, 1:11). During mating attempts with stoppled females or after a successful shield removal, the picking motion of the valvae near the copulatory opening (**Video 4**, 1:29; Video 2, 1:27) suggests that males use them to remove large stopples or any device remaining in the vagina. In addition, the aedeagus may serve as an auger to penetrate besides or through internal plugs or unshielded stopples as well as a lever to remove stopples (Orr 1995).

We observed that the valvae are conspicuous due to their relatively large size and sparse scale coverage, unlike many butterflies' (e.g. **Figure 29**A–H). They also bald over time (**Figure 30**A, B, D, E), probably due to intensive use. Large, conspicuous valvae may indicate their special role in sphragis removal, compared to other, also sphragis-bearing species (e.g. *Parnassius davydovi*, **Figure 29**E). The valvae bear coarse bristles, especially at the posterior tips (**Figure 30**A–F), even on old males, when scales had already been lost. Bristles might bear mechano- and/or olfactory receptors (Prof. Jean-François Picimbon, pers. comm.), likely crucial in identifying

CAPs in the female copulatory opening, directing the movement of the valvae and informing the male whether to persist or abandon the female.

Figure 29. Valvae in the Clouded Apollo (A) and in a few other butterfly species. *Parnassius mnemosyne* (A), *Zerynthia polyxena*, D&S. (B), *Papilio machaon*, L. (C), *Iphiclides podalirius*, L. (D) and *Parnassius davydovi*, C. (E) belong to the family Papilionidae (A–E); *Pieris rapae*, L. (Pieridae; F), *Minois dryas*, S. (Nymphalidae; G) and *Argynnis paphia*, L. (Nymphalidae; H). *Parnassius mnemosyne* (A) belongs to the *Driopa* and *P. davydovi* (E) to the *Kailasius* subgenus (Condamine et al. 2018, Zheng et al. 2018). *P. davydovi* (E) is distributed in Kyrgyzstan (Churkin 2006), while the other species (A–D, F–H) are common in Hungary. Butterflies other than *Parnassius davydovi* were caught in the vicinity of the study site. Photos were taken by JK, except E (ÁG).

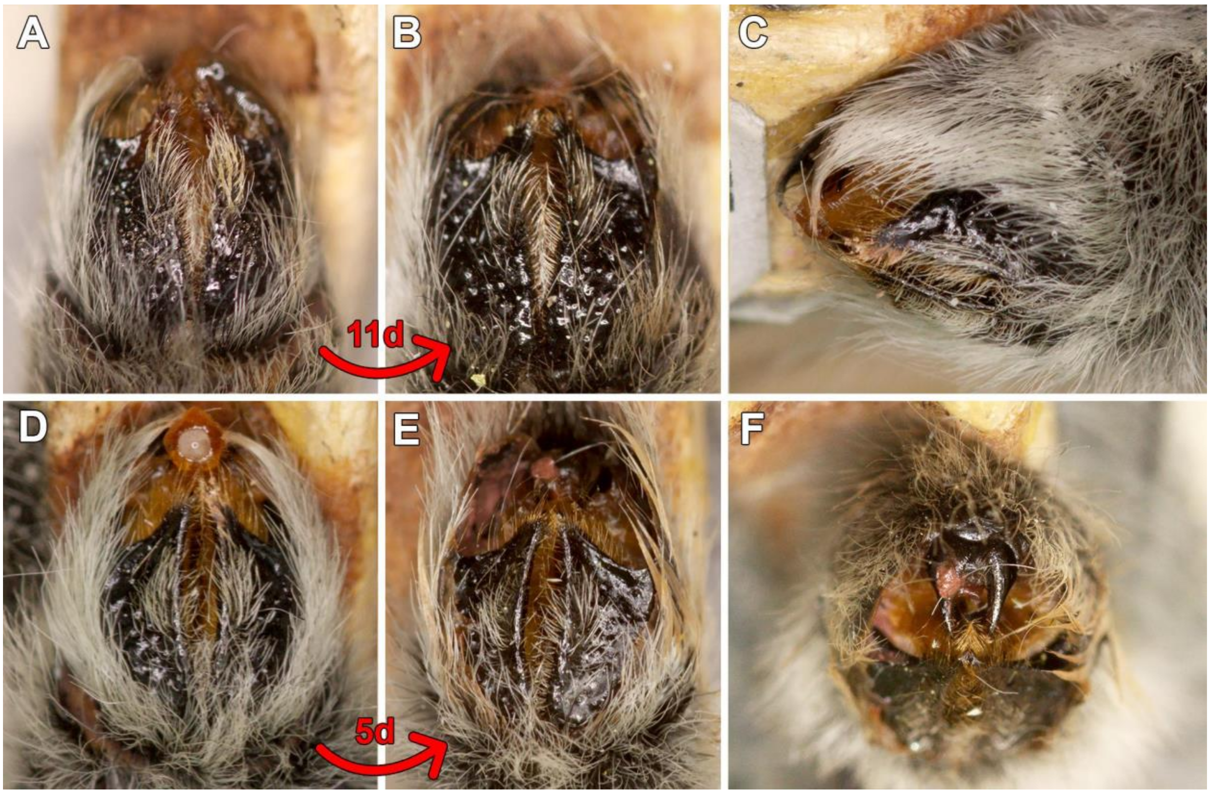


Figure 30. Valvae (A–D) and unci (F) in Clouded Apollos. Ventral view (A–B, D–E), lateral view (C) and posterior view (F). Photographs on two different males; male1 (A–C), male2 (D–F). Eleven days elapsed between the two photographs were taken in male1 (A–B), 5 days in male2 (D–E). Note that light grey hairs become scarcer over time on the same valvae, making the brownish-yellow bristles along the medial and posterior edges more visible. Bristles can also be seen from the posterior view on the tip of the blurred valvae, just below the unci (F). Photos were taken by JK.

5.5. CAP-variation

Scarce shield losses (**Table 6**) relative to the total number of shields and to the number of small CAP losses imply that removing shields is more costly than removing small CAPs. Shield removals may last up to several hours, while once the shield is severed, the remaining plug is removed within a few minutes, although we have not enough data on the length of these processes to provide reliable estimates. We also assume that shield removal imposes high energetic costs on males, including holding the female in position (**Video 4**, 0:00). Shield dimension measurements show that there is a considerable variation among shields in length, height and width with some extremities. Sources of this variation may be variance in male resources (Matsumoto 1987, Orr 2002), male decision on investment or disturbance either by rival males, unwilling females, or predators. Although we rarely observed matings and could not relate male size to the shields' they produced, Pásztor et al. (2022) found large variance in male thorax width in the same population as this study. Thorax width is related to the amount of flight muscles (Stjernholm et al. 2005) and might also be related to male capacity in producing CAPs.

The shape and size of the sphragis may play an important role in sphragis removal attempts, since larger shields may be more difficult to grab with the valvae and the neck of the stopple could be harder to reach. Shield dimensions were weakly correlated, if at all (**Figure 20**), indicating a lack of strong material trade-off among the dimensions when producing a sphragis. This could be because males form the shield with the help of their valvae, therefore dimensions could be partly dependent on valvae sizes and/or males may decide, upon female quality, on which CAP-type to produce or how large a shield should be. Smaller female insects within a population have generally lower fecundity and produce lower quality offspring (Honěk 1993, Sibley et al. 2001). Therefore, if males are able to assess female quality, they would benefit from the decision on investing in different CAP-types or shield sizes.

We also found variation in shield transparency and colour among shields and within a single shield (**Figure 22**A–I), as some shields had, for example, darker anterior and lighter posterior parts (**Figure 22**C). Moreover, others seemed to slightly change colour over time. Colouration may depend on wall thickness (**Figure 22**C, E), as well as the currently available nectar sources influencing the different materials used to build a shield and the always varying weather conditions (e.g. humidity) impacting the water content of the sphragides.

5.6. CAP-type and size

We found that shields were more common and much less prone to be lost than small CAPs and usually persisted during the whole life of females. This indicates that shields were more efficient devices in securing paternity than small CAPs. Furthermore, longer shields were more prone to persist than shorter ones. These suggest that (i) the more a male invests in CAPs, the more prone it is to secure paternity and (ii) the final outcome of CAP-production (i.e. CAP-type and size) takes place on a more or less continuous scale of investment. The resources males can actually allocate in mate-guarding may heavily impact success, and this would in turn depend on actual male quality, such as body size (Schöfl & Taborsky 2002), age (Pásztor et al. 2022), as well as body reserves (Stjernholm & Karlsson 2000). Male investment is supposed to be constrained through production capacity. ‘Incomplete sphragides’, structures attached to the female copulatory opening, but reduced in size compared to the species-specific sphragis (Carvalho et al. 2019), likely equivalent or similar to what we denote as small CAPs, were produced by males after consecutive matings and attributed to male resource depletion in several butterfly species (Carvalho et al. 2017, 2019, Matsumoto 1987, Orr 1988).

5.7. Female quality

Male decision on investment should theoretically depend on female quality, the expected residual reproductive value of the female at the time of mating and the male’s expected share of its prospective progeny (Bonduriansky 2001, Fischer et al. 2008). Males may be able to assess if females are virgin or already mated, and invest accordingly. Its mechanism might be straightforward in the case of female Clouded Apollos with CAPs, i.e. anything blocking the vagina means the female has already mated. We have no information if males were able to assess if no CAP females were virgin, and our methods are inappropriate to ascertain female virginity.

Females with both larger actual size (i.e. wider thoraces relative to head width), and larger natal size (i.e. wider heads) were more prone to receive shields than smaller females, while body mass, wing and proboscis length were not related to the received CAP-types. Thorax size is also related to the actual amount of flight muscle mass (Stjernholm et al. 2005) and flight muscle mass is related to fecundity, since butterflies are able to allocate nitrogen from thoracic muscles into reproduction or into somatic maintenance (Karlsson 1998, Stjernholm et al. 2005). Investment in mate-guarding increased with increasing female size (Amphipoda: Dick & Elwood 1990, Crustaceans: Jormalainen 1998, Amphipoda: Takeshita & Henmi 2010) and large females were chosen over small ones in different arthropod species (Coeloptera: Knox & Scott 2006, Isopoda: Manning 1975, Pericarida: Shuster 1981, Isopoda: Thompson & Manning 1981, but see Lepidoptera: Jarrige et al. 2016, Orthoptera: Mahoney et al. 2017, Heteroptera: Schöfl & Taborsky 2002). Since larger female insects were supposed to be better quality (Gilbert 1984, Honěk 1993, Oberhauser 1997, Okada et al. 2021, Prenter et al. 1994, Sibley et al. 2001), i.e. have higher fecundity, males should benefit from investing more in guarding larger females (e.g. Knox & Scott 2006). Thus, larger females may receive shields due to their higher residual reproductive value and because shields are more efficient in securing paternity. These imply male preference for large females, size-dependent investment into guarding and an unknown mechanism of size assessment.

Males do not court females in Clouded Apollos but force copulations, similarly to many other sphragis-producing species (Kawagoe et al. 2001, Matsumoto et al. 2018, McCorkle & Hammond 1985, Orr & Rutowski 1991, Orr 1988, 1995, 1999, 2002). We observed that females often tried to resist mating in the first couple of minutes while the males tried to grab them and positioned themselves for mating. Such struggles might serve as quality assessment relative to partner quality through their strengths, for both males and females. We hypothesise that males may decide on investment, while females on how long they allow the male to mate, and that these together would determine the final quality and size of the mate-guarding device. During some of the few field observations where we could see mating pairs before and during separation, the female moved first after a long motionless ‘mating triangle’ position (**Figure 13**A–C), as if it initiated separation. This suggests that females might have some control at least in some of the cases in terminating mating and CAP-production, ultimately constraining CAP-type and size. Female control over copulation duration was reported but in different mating systems, in water striders (*Gerris buenoi*, Heteroptera: Gerridae; Rowe 1992) and redback spiders (*Latrodectus hasselti*, Arachnida: Theridiidae; Andrade et al. 2005). Interestingly, in a sphragis-bearing papilionid butterfly, males were unable to force copulations on females with much longer wings than their own, suggesting active female mate choice (*Cressida cressida*, Lepidoptera: Papilionidae; Orr 1999). If it was also true for Clouded Apollos, mating females would have to decide on copulation duration with good-enough quality males only, since low-quality males could not even mate. A proportion of males may not mate in protandrous lepidopterans with male-biased sex ratios and high male-male competition (Bennik et al. 2020). Note that male investment upon female size cannot be concluded here definitively, since female size may vary over age and the timing of entry to the population (Pásztor et al. 2022), confounders we could not control for in this study (see also 5.9. Dynamic changes section below).

5.8. Shield-to-CAP ratio and adult sex ratio

Shield-to-CAP ratio and adult sex ratio both decreased over the flight periods (**Figure 28**). Similarly, studies investigating the presence or lack of sphragis (shield) found females lacking a sphragis more frequently at the end of the flight period than earlier (*Parnassius mnemosyne*, Vlašánek et al. 2009, Vlašánek & Konvička 2009; *Parnassius smintheus*, Matter et al. 2004, Roland et al. 2000, but see Auckland et al. 2004 for *Parnassius clodius*). Calabrese et al. (2008) explained the lack of sphragis at the end of the reproductive period with the lack of capable males, termed as matelessness, as ASRs become more and more female-biased. However, sampling (Auckland et al. 2004, Matter et al. 2004, Roland et al. 2000) upon which this explanation was based, did not take into account small CAPs that might occur in those species as well. Our results do not refute the female matelessness hypothesis, rather they emphasise that the lack of a sphragis (shield) does not inform on matelessness. As in many lepidopterans (Teder et al. 2021), all studied *Parnassius* populations were protandrous (Calabrese et al. 2008, Szigeti et al. 2019, Vlašánek et al. 2009, Vlašánek & Konvička 2009). These imply strongly male-biased populations at the beginning, less male-biased or even female-biased at the end of the flight period, as were also found in another Clouded Apollo population (Vlašánek et al. 2009, Vlašánek & Konvička 2009). This, together with both sexes living in the flight period later being smaller than those living earlier (Pásztor et al. 2022), thus probably having less reserves, imply that female matelessness might occur in this population at the very end of the flight period.

The seasonal decline of shield-to-CAP ratios followed the decline of adult sex ratios with a three-day lag (**Figure 9**, **Figure 28**). The main source of this bias in actual ASR is likely protandry, but further bias towards males may also be because there are more males than females in the Clouded Apollo (unpublished data for a different population: **Table 7**, Leánykúti-rét; Vlašánek et al. 2009, Vlašánek & Konvička 2009), as well as in other *Parnassius* populations studied so far (Vlašánek et al. 2009; see also references therein). The intensity of competition for mating among males is expected to be higher with a stronger male-bias within the population (Kvarnemo & Ahnesjö 2002, Weir et al. 2011). Male competition for mating then increases with male ratio, which may result in increased benefits from mate guarding (Alcock 1994). In crustaceans, investment in guarding increased with the increasing male ratio (Dick & Elwood 1990, Jormalainen 1998, Takeshita & Henmi 2010). Last male sperm precedence (Alcock 1994, Boggs & Watt 1981, Clarke & Sheppard 1962, Labine 1966, Parker 1970, Simmons 2001, Sims 1979, but see Tregenza & Wedell 2002), not investigated in Clouded Apollos but common in insects, may further explain why males are less prone to invest in shields later in the flight period, when competition for mating is low, and in consequence the risk of small CAP-removal is also likely to be relatively low.

We investigated adult sex ratios (ASR). In contrast, Alcock (1994) and Kvarnemo & Ahnesjö (2002) suggested that operational sex ratios (OSR), i.e. the ratios of males to females willing and able to mate at an actual time point, will determine the measure of competition, and ultimately, the benefit from mate-guarding. In the case of Clouded Apollos, CAP-type and size influence female ability to mate, i.e. its 'time-in' period (Kvarnemo & Ahnesjö 2002), therefore OSR. We do not have any measurable cues to estimate 'time-in' or 'time-out' for males, thus the gap between ASR and OSR estimates cannot be assessed. We predict that ASR underestimates OSR early in the flight period when young, unmated males are abundant and most mated females are shielded. We are unable to predict the ASR-OSR relationship for the late flight period, with both sexes ageing and females bearing small CAPs being more frequent.

How males could assess the intensity of competition is unknown. One potential explanation would be that patrolling males' encounter rates with competitors and entirely (no CAP) as well as partially (small CAP) available females may influence male decision. Our results suggest that males may not be able to immediately assess the level of competition for mating. Matings usually last several hours and may easily take half a day. This explains a part of the three-day time lag between shield-to-CAP and adult sex ratios, but we are still far from understanding it entirely.

5.9. Dynamic changes

The major limitation of this observational study is that we are not able to discern the impacts of different variables changing over the flight period. In the same population as the present study, Clouded Apollo thorax width declined with age in both sexes, and individuals emerging late in the flight period tended to be smaller (Pásztor et al. 2022). Both ageing and small body size are related to less reserves (Boggs & Inouye 2012, Vande Velde et al. 2013) and probably incur low mobility. These may reduce foraging for both sexes, egg laying activity for females and patrolling and investing in CAPs for males. These imply that females late in the flight period had lower residual reproductive values than those flying early, partly because of ageing, partly because they emerged later, e.g. with a poorer initial condition (Pásztor et al. 2022). Furthermore, females with narrower thoraces had higher risk to disappear at a given time point from the population than wider females. Conclusions on the time-period of a state cannot be directly drawn from the multistate model, i.e. higher risk to disappear at a given time point is not equivalent to shorter presence in the population; the latter depends on the entire event history. In our model, no transitions were allowed from the state disappear (**Figure 25**), since disappear meant that a female was not observed any more in the population. This supports the hypothesis that females with narrower thoraces disappear sooner than wider females. Disappearance may be due to emigration, undetectability (lower activity), or death. Also, females entering the population late in the flight period had shorter observation periods ($\rho = -0.12$, $p < 0.009$, n = 492 all years combined, *Spearman's rank correlation test*; **Figure 31**). We assume that observation periods are associated with lifespan, thus short-living small females had less time to lay eggs. Males of the Mediterranean Flour Moth (*Ephestia kuehniella,* Lepidoptera: Pyralidae) invested more in larger and younger females than in smaller and older ones (Xu & Wang 2009). In Clouded Apollos, males flying later in the flight period were probably more constrained on investing into CAPs for the same reasons as female quality deterioration, as well as body reserve depletion (Stjernholm & Karlsson 2000). Depletion due to resources used for CAP-production over consecutive matings of an individual was found in other papilionid species in laboratory conditions (Matsumoto 1987, Niihara & Watanabe 2009, Orr 2002). All these dynamic changes are likely to influence operational sex ratios, therefore the level of competition for mating in males and the actual investment decisions, ultimately the CAP-types produced.

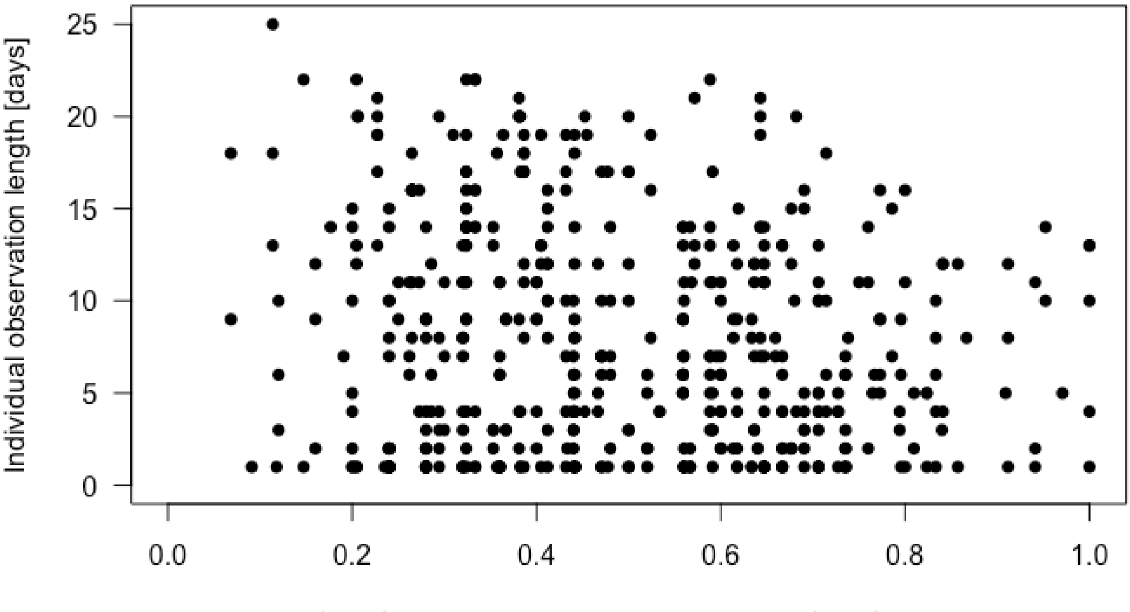


Figure 31. Individual observation length over the flight period between 2015 and 2020 in Clouded Apollos. The x-axis represents days when individuals were first observed.

5.10. Constrained polyandry

The females of this Clouded Apollo population are potentially polyandrous, regardless of their willingness for multiple mating or disability to resist males that force copulations. Since most females receive shields, probably at their first mating, and shields most often persist over life preventing future matings, shields are likely an important component of reproductive load, the cost imposed on reproductive success for both sexes by male-male competition for mating (Holland & Rice 1999, Okada et al. 2021) and severely constrain polyandry. In consequence, most females, probably especially the larger, younger and the ones flying at an early phase of the flight period, are (i) deprived of postcopulatory female choice and (ii) their progeny have reduced genetic diversity compared to multiply mated females. Postcopulatory female choice was found to enhance reproductive success in a wide range of taxa, including insects (Firman et al. 2017). Depriving females of this opportunity indicates a high level of intersexual conflict and an evolutionary phase when females seem to be losing to males in the arms-race. However, this would be mitigated if Clouded Apollo females were able to reject poor quality males as in the papilionid butterfly *Cressida cressida* (Lepidoptera: Papilionidae; Orr 1999). Multiple matings may also increase female fitness through enhanced genetic variability in their offspring compared to monogamous females through bet-hedging, i.e. in a fluctuating environment, at least some of the offspring would likely to survive (Jennions & Petrie 2000). This in turn might severely affect populations transitioning from relatively stable to unstable environments caused by climate change-driven unpredictable weather conditions or habitat change by extensive forest management.

5.11. Male costs and benefits

Based on our results, we assume that producing a shield is much more costly than producing small CAPs due to the differences in the amount of material and production time these CAPs require. We could not estimate how many shields a male can produce, but male depletion resulting in 'incomplete sphragis' observed under laboratory conditions and that species producing larger sphragides produce smaller spermatophores in various sphragis-bearing butterflies indicate high costs of large sphragides (Matsumoto & Suzuki 1995, Orr 1995). In addition, the time spent on producing (or removing) a shield cannot be spent on finding consecutive mates or replenishing resources by feeding (Alcock 1994). An extremely rare cost for males is that a shield may be stuck around its valvae in the course of production (**Figure 14**A–C) that might impede the production of a subsequent shield by blocking the orifices through which CAP material is released from the male body. The position of the shield implies that it was stuck on the male when produced, rather than imposed on it by another male.

We observed a few males and females mating several times. In such promiscuous mating systems, strong male-male competition for mating and sexual dimorphism is expected to result in strong sexual conflict (Parker 1979), especially if CAPs impede oviposition (**Figure 15**A–D). Annual variation in the proportion of CAPs (**Table 4**) implies fluctuating conditions that influence male payoffs from producing a specific CAP-type. This can be caused by the annual variation in adult sex ratios (as found in a different habitat: Bella 2017, Leánykúti-rét, **Table 7**) inducing different degrees of male competition. The proportion of shields varied across years (**Table 4**) but was always much higher than the proportion of filaments and stopples combined. If the last male fertilises most of a multiply mated female's eggs (last male sperm precedence, common in insects; (Boggs & Watt 1981, Clarke & Sheppard 1962, Labine 1966, Parker 1970, Simmons 2001, Sims 1979, but see Tregenza & Wedell 2002), those able to monopolise a female for the rest of its life, may incur a selective advantage over rivals (Alcock 1994). However, we did not perform any genetic studies, neither count spermatophores considering last male precedence in this species. As in other studies (*Parnassius mnemosyne*, Vlašánek & Konvička 2009; other *Parnassius* species, Auckland et al. 2004, Calabrese et al. 2008, Matter et al. 2012), our results also show that most females were shielded (**Table 4**), often for life, implying that larger male investment was more efficient, and in general, required for securing paternity. However, males may either be constrained or unwilling to invest so much in their partners in some of the cases.

5.12. Female costs and benefits

Females may also benefit from being capped, if shields and large stopples serve as visual (Dickinson & Rutowski 1989, Drummond 1984, Orr & Rutowski 1991, Orr 1995, Saigusa 1973) and tactile cues as deterrents (Matsumoto 1987, Orr 1988), protecting them from male harassment. In most cases, males were observed abandoning females immediately after noticing the sphragis. However, in a few cases, multiple males were observed grabbing the same female with the legs, fighting each other and using the valvae to hold on the female abdomen, and this might cause injuries in the female. Shields may provide mechanical protection against potential injuries from the males' attempt to dig out stopples with the sharp aedeagus and valvae. Males reportedly injured females during mating in a handful of insects (Rönn et al. 2007, Stutt & Siva-Jothy 2001). Escaping harassment also saves time that can be spent on foraging, basking or egg laying, and might save energy for the female. We have very few observations on mating and CAP-removal. Females occasionally dragged the males when crawling in the vegetation as if they were trying to get rid of the male (Video 2, 1:17). However, this interpretation may not necessarily be true: although females try to carry the male to conspicuous places, in other cases the female heads into dense vegetation, as if it would try to hide themselves. Although female control over copulation duration was reported in the water strider (*Gerris buenoi*, Heteroptera: Gerridae; Rowe 1992), this is likely not such a clear case in Clouded Apollos. The large shield likely provides more protection against potential harassment than small CAPs.

In contrast, sphragides are large and heavy devices (up to 3–5% of the female body mass) which may cause higher wing loading (Fric et al. 2006) and longer handling time during egg laying. Large shields may impede egg laying: we found eggs stuck to the shield that could not be laid (**Figure 15**A–D). It is not necessarily a fertility cost if larvae can hatch from stuck eggs with a similar probability as from eggs glued on female-chosen surfaces such as litter or grass and if the female dies around *Corydalis* plants. However, large shields may make it more difficult to find a suitable surface with the ovipositor, thus increasing the temporal cost of oviposition. Interestingly, in a different population in Sweden, Clouded Apollos were observed dropping their eggs (Wiklund 1984) instead of gluing them to a surface, that lessens the cost of oviposition, especially for sphragis-bearers. This implies that caterpillars are able to hatch from eggs not attached to a surface (also Orsolya Dombi pers. comm. for a Hungarian population; Börzsöny, **Table 7**). Furthermore, the reduced genetic variance in the offspring of females capped for life might incur fitness costs due to less likely survivors among a more homogeneous lot of progeny compared to females that are able to mate with several males, especially in highly fluctuating environments (Jennions & Petrie 2000). Female fertility declined in flour beetles (*Gnatocerus cornutus,* Coleoptera: Tenebrionidae) with increasing male-male competition in an experimental evolution setup; natural selection against sexually selected male exaggerated traits reduced exaggeration and increased female fertility (Okada et al. 2021).

6. Conclusion

Carvalho et al. (2019) reported the regular occurrence of ‘incomplete sphragides’ in several *Pteronymia* species (Lepidoptera: Nymphalidae). To our best knowledge, our study is the first to demonstrate that besides large sphragides, males regularly produce smaller devices to cap female parnassian butterflies. We assume that CAP-size is proportional to its effectiveness preventing females from future matings, as well as to costs the males incur when investing. Direct observations and video recordings indicate different and multiple roles of the valvae and the aedeagus in producing, fastening and removing CAPs. We predict that alternative mate-guarding devices similar to that of *P. mnemosyne* could be found under scrutiny in more species known for sphragis bearing.

Furthermore, this multi-year study is unique in investigating male investment into mate-guarding, female remating and its association with female size and adult sex ratio in a natural insect population. In Clouded Apollos, shields were the most frequent mate-guarding devices and were more persistent than the smaller stopples or filaments, i.e. small CAPs. Presumably, due to their larger size, shields are much more costly to produce and fix on the female than small CAPs. The net benefits from shields compared to small CAPs seemed to decline over the progress of the flight period. We assume that the final outcome of CAP production, i.e. the CAP-type produced and the size of the CAP depends also on the relative quality of the mates at an actual mating attempt, as well as operational sex ratios and the progress of the flight period. We propose that at high male-male competition, if a male can afford, produces a shield, but if medium competition occurs, produces a shield for a large female or a small CAP for a small female and if competition is low, produces a small CAP. Future experimental studies should test these assumptions and potential scenarios to discern the role of body size, reserve depletion, ageing and the expected residual lifespan of the parties, as well as operational sex ratio. Investigating the capacity of females to control mating duration, and in consequence CAP-type and size, would also be essential to understand the dynamics of CAP-production. ‘High quality’ females were more deprived of postcopulatory female choice and genetic variance in their offspring may be reduced compared to the ‘low quality’, therefore more polyandrous females. Addressing the potential costs and benefits associated with this pattern would further enhance our understanding of the evolution of mating systems with CAP-production.

7. New scientific results

1. We found that besides large sphragides, smaller, alternative mate-guarding devices had regularly been produced by males within a butterfly species. This is the first evidence in the genus *Parnassius*.
2. We found considerable variation in the size and shape of all mate-guarding devices.
3. We provide photographic and video evidence on mate-guarding device removals.
4. We found that multiple and different types of mate-guarding devices may occur consecutively on females.
5. The persistence of mate-guarding devices on the females increased with increasing male material investment in these devices.
6. The size and type of the male produced mate-guarding devices were associated with female size and adult sex ratio (ASR).

8. References

Alberts, S. C., Altmann, J., Wilson, M. L.: **Mate guarding constrains foraging activity of male baboons**, Animal Behaviour, 51(6). 1269–1277, 1996. https://doi.org/10.1006/anbe.1996.0131

Alcock, J.: **Postinsemination associations between males and females in insects: the mate-guarding hypothesis**, Annual Review of Entomology, 39. 1–21, 1994.https://doi.org/10.1146/annurev.ento.39.1.1

Andersson, M.: **Sexual Selection***,* (Vol. 72), Princeton University Press, 1994.

Andersson, M., Simmons, L. W.: **Sexual selection and mate choice**, Trends in Ecology Evolution, 21(6). 296–302, 2006. https://doi.org/10.1016/j.tree.2006.03.015

Andrade, M. C. B., Gu, L., Stoltz, J. A.: **Novel male trait prolongs survival in suicidal mating**, Biology Letters, 1(3). 276–279, 2005. https://doi.org/10.1098/RSBL.2005.0318

Areja-Gavina, M. K. D., Torres, M. C., Gamilla, G. B., Sakaguchi, T., Ito, H., Rabajante, J. F., Tubay, J. M., Yoshimura, J., Morita, S.: **Exaggerated evolution of male armaments via male–male competition**, Ecology and Evolution, 11(11). 6977–6992, 2021. https://doi.org/10.1002/ece3.7546

Arnqvist, G., Rowe, L.: **Antagonistic coevolution between the sexes in a group of insects**, Nature, 415(6873). 787–789, 2002. https://doi.org/10.1038/415787a

Arnqvist, G., Rowe, L.: **Sexual Conflict***,* (Vol. 31), Princeton University Press, 2005.

Auckland, J. N., Debinski, D. M., Clark, W. R.: **Survival, movement, and resource use of the butterfly *Parnassius clodius***, Ecological Entomology, 29(2). 139–149, 2004. https://doi.org/10.1111/j.0307-6946.2004.00581.x

Bateman, A. J.: **Intra-sexual selection in *Drosophila***, Heredity, 2(3). 349–368, 1948.

Bell, G.: **The evolution of anisogamy**, Journal of Theoretical Biology, 73(2). 247–270, 1978. https://doi.org/10.1016/0022-5193(78)90189-3

Bella, M.: **Kis Apolló-lepke ivararány változásának dinamikája [Seasonal sex ratio dynamics in Clouded Apollo butterflies]***,* BSc thesis in Hungarian, University of Veterinary Medicine Budapest, 2017.

Bennik, R. M., Hoare, R. J. B., Holwell, G. I.: **Seasonal variation in body size and male mating success within lichen tuft moths Izatha (Lepidoptera: Xyloryctidae)**, Austral Entomology, 59(4). 802–809, 2020. https://doi.org/10.1111/AEN.12495

Benvenuto, C., Weeks, S. C.: **Intersexual conflict during mate guarding in an androdioecious crustacean**, Behavioral Ecology, 23(1). 218–224, 2012. https://doi.org/10.1093/beheco/arr178

Bergström, A.: **Oviposition site preferences of the threatened butterfly *Parnassius mnemosyne* - Implications for conservation**, Journal of Insect Conservation, 9(1). 21–27, 2005. https://doi.org/10.1007/s10841-004-3204-4

Birkhead, T. R.: **Sperm competition: evolutionary causes and consequences**, Reproduction, Fertility and Development, 7(4). 755–775, 1995. https://doi.org/10.1071/rd9950755

Birkhead, T. R., Pizzari, T.: **Postcopulatory sexual selection**, Nature Reviews Genetics, 3(4). 262–273, 2002. https://doi.org/10.1038/nrg774

Boggs, C. L., Inouye, D. W.: **A single climate driver has direct and indirect effects on insect population dynamics**, Ecology Letters, 15(5). 502–508, 2012. https://doi.org/10.1111/j.1461-0248.2012.01766.x

Boggs, C. L., Watt, W. B.: **Population structure of pierid butterflies IV. Genetic and physiological investment in offspring by male *Colias***, Oecologia, 50(3). 320–324, 1981. https://doi.org/10.1007/BF00344970

Bonduriansky, R.: **The evolution of male mate choice in insects: a synthesis of ideas and evidence**, Biological Reviews, 76(3). 305–339, 2001. https://doi.org/10.1017/S1464793101005693

Bryk, F.: **Grundzuege der sphragidologie**, Arkiv För Zoologi, 11. 1–38, 1918.

Bryk, F.: **Bibliotheca sphragidologica**, Archiv Für Naturgeschichte, 85. 102–183, 1919.

Calabrese, J. M., Ries, L., Matter, S. F., Debinski, D. M., Auckland, J. N., Roland, J., Fagan, W. F.: **Reproductive asynchrony in natural butterfly populations and its consequences for female matelessness**, Journal of Animal Ecology, 77(4). 746–756, 2008. https://doi.org/10.1111/j.1365-2656.2008.01385.x

Canales-Lazcano, J., Contreras-Garduño, J., Cordero, C.: **Strategic adjustment of copulatory plug size in a nematode**, Current Zoology, 65(5). 571–577, 2019. https://doi.org/10.1093/cz/zoy067

Carey, J. R., Liedo, P., Müller, H. G., Wang, J. L., Vaupel, J. W.: **Dual modes of aging in Mediterranean fruit fly females**, Science, 281(5379). 996–998, 1998. https://doi.org/10.1126/SCIENCE.281.5379.996

Carey, J. R., Papadopoulos, N., Kouloussis, N., Katsoyannos, B., Müller, H. G., Wang, J. L., Tseng, Y. K.: **Age-specific and lifetime behavior patterns in *Drosophila melanogaster* and the Mediterranean fruit fly, *Ceratitis capitata***, Experimental Gerontology, 41(1). 93–97, 2006. https://doi.org/10.1016/J.EXGER.2005.09.014

Carvalho, A. P. S., Mota, L. L., Kawahara, A. Y.: **Intersexual ‘Arms Race’ and the evolution of the sphragis in *Pteronymia* butterflies**, Insect Systematics and Diversity, 3(1). 1–13, 2019. https://doi.org/10.1093/isd/ixy021

Carvalho, A. P. S., Orr, A. G., Kawahara, A. Y.: **A review of the occurrence and diversity of the sphragis in butterflies (Lepidoptera, Papilionoidea)**, ZooKeys, 694. 41–70, 2017. https://doi.org/10.3897/zookeys.694.13097

Charlesworth, D., Charlesworth, B.: **Evolutionary biology: the origins of two sexes**, Current Biology, 20(12). 519–521, 2010. https://doi.org/10.1016/j.cub.2010.05.015

Churkin, S.: **A new species of *Parnassius* Latreille, 1804, from Kyrgyzstan (Lepidoptera, Papilionidae)**, Helios, 7. 142–158, 2006.

Clarke, C. A., Sheppard, P. M.: **Offspring from double matings in swallowtail butterflies**, The Entomologist, 95. 199–203, 1962.

Clutton-Brock, T. H.: **Review Lecture: Mammalian mating systems**, Proceedings of the Royal Society of London. Series B: Biological Sciences, 236(1285). 339–372, 1989. https://doi.org/10.1098/rspb.1989.0027

Condamine, F. L., Rolland, J., Höhna, S., Sperling, F. A. H., Sanmartín, I.: **Testing the role of the Red Queen and Court Jester as drivers of the macroevolution of Apollo butterflies**, Systematic Biology, 67(6). 940–964, 2018. https://doi.org/10.1093/sysbio/syy009

Cueva Del Castillo, R.: **Body size and multiple copulations in a neotropical grasshopper with an extraordinary mate-guarding duration**, Journal of Insect Behavior, 16(4). 503–522, 2003. https://doi.org/10.1023/A:1027303323242

Dahlsjö, C. A. L.: **Head width–body mass equation: facilitating worker termite biomass estimates**, Ecological Entomology, 41(6). 741–744, 2016. https://doi.org/10.1111/EEN.12349

Danzy, J., Gutierrez, V., Pampush, J., Campbell, B.: **Factors affecting the distribution of copulatory plugs in rhesus monkeys *(Macaca mulatta)* on Cayo Santiago**, Folia Primatologica, 80(4). 264–274, 2009. https://doi.org/10.1159/000252585

Davies, N. B.: **Cooperation and conflict among dunnocks, *Prunella modularis*, in a variable mating system**, Animal Behaviour, 33(2). 628–648, 1985. https://doi.org/10.1016/S0003-3472(85)80087-7

de Wreede, L. C., Fiocco, M., Putter, H.: **mstate: an R package for the analysis of competing risks and multi-state models**, Journal of Statistical Software, 38(7). 1–30, 2011. https://doi.org/10.18637/JSS.V038.I07

Dick, J. T. A., Elwood, R. W.: **Symmetrical assessment of female quality by male *Gammarus pulex* (Amphipoda) during struggles over precopula females**, Animal Behaviour, 40(5). 877–883, 1990. https://doi.org/10.1016/S0003-3472(05)80989-3

Dickinson, J. L., Rutowski, R. L.: **The function of the mating plug in the chalcedon checkerspot butterfly**, Animal Behaviour, 38(1). 154–162, 1989. https://doi.org/10.1016/S0003-3472(89)80074-0

Dixson, A. F., Anderson, M. J.: **Sexual selection, seminal coagulation and copulatory plug formation in primates**, Folia Primatologica, 73(2–3). 63–69, 2002. https://doi.org/10.1159/000064784

Drummond, B. A.: **Multiple mating and sperm competition in the Lepidoptera**, In: *Sperm Competition and the Evolution of Animal Mating Systems*, R. L. Smith (Ed.), Academic Press, 1984. https://doi.org/10.1016/b978-0-12-652570-0.50016-6

Dunham, A. E., Rudolf, V. H. W.: **Evolution of sexual size monomorphism: the influence of passive mate guarding**, Journal of Evolutionary Biology, 22(7). 1376–1386, 2009. https://doi.org/10.1111/j.1420-9101.2009.01768.x

Eberhard, W. G.: **Sexual Selection and Animal Genitalia***,* Harvard University Press, 1985.

Eberhard, W. G.: **Female control: sexual selection by cryptic female choice***,* (Vol. 69), Princeton University Press, 1996.

Eltringham, H.: **III. On the source of the sphragidal fluid in *Parnassius apollo* (Lepidoptera)**, Transactions of the Royal Entomological Society of London, 73(1–2). 11–15, 1925. https://doi.org/10.1111/J.1365-2311.1925.TB02859.X

Emlen, D. J.: **The evolution of animal weapons**, Annual Review of Ecology, Evolution, and Systematics, 39(1). 387–413, 2008. https://doi.org/10.1146/annurev.ecolsys.39.110707.173502

Fedorka, K. M., Mousseau, T. A.: **Material and genetic benefits of female multiple mating and polyandry**, Animal Behaviour, 64(3). 361–367, 2002. https://doi.org/10.1006/ANBE.2002.3052

Firman, R. C., Gasparini, C., Manier, M. K., Pizzari, T.: **Postmating female control: 20 years of cryptic female choice**, Trends in Ecology Evolution, 32(5). 368–382, 2017. https://doi.org/10.1016/J.TREE.2017.02.010

Fischer, K., Perlick, J., Galetz, T.: **Residual reproductive value and male mating success: older males do better**, Proceedings of the Royal Society of London. Series B: Biological Sciences, 275(1642). 1517–1524, 2008. https://doi.org/10.1098/RSPB.2007.1455

Fric, Z., Klimova, M., Konvička, M.: **Mechanical design indicates differences in mobility among butterfly generations**, Evolutionary Ecology Research, 8. 1511–1522, 2006.

Gavrilets, S., Arnqvist, G., Friberg, U.: **The evolution of female mate choice by sexual conflict**, Proceedings of the Royal Society of London. Series B: Biological Sciences, 268(1466). 531–539, 2001. https://doi.org/10.1098/rspb.2000.1382

Gergely, P., Gór, Á., Hudák, T., Ilonczai, Z., Szombathelyi, E.: **Nappali lepkéink – Határozó terepre és természetfotókhoz (Második, javított kiadás) – A Field Guide to the Butterflies of Hungary (Second, corrected edition)***,* Kitaibel Kiadó, 2018.

Gilbert, N.: **Control of fecundity in *Pieris rapae*: I. The problem**, The Journal of Animal Ecology, 53(2). 581–588, 1984. https://doi.org/10.2307/4536

Hagen, M., Dupont, Y. L.: **Inter-tegular span and head width as estimators of fresh and dry body mass in bumblebees (*Bombus* spp.)**, Insectes Sociaux, 60(2). 251–257, 2013. https://doi.org/10.1007/S00040-013-0290-X

Holland, B., Rice, W. R.: **Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load**, Proceedings of the National Academy of Sciences of the United States of America, 96(9). 5083–5088, 1999. https://doi.org/10.1073/PNAS.96.9.5083

Honěk, A.: **Intraspecific variation in body size and fecundity in insects: a general relationship**, Oikos, 66(3). 483–492, 1993. https://doi.org/10.2307/3544943

Hothorn, T., Bretz, F., Westfall, P.: **Simultaneous inference in general parametric models**, Biometrical Journal, 50(3). 346–363, 2008. https://doi.org/10.1002/bimj.200810425

Janicke, T., Fromonteil, S.: **Sexual selection and sexual size dimorphism in animals**, Biology Letters, 17(9). 1–16, 2021. https://doi.org/10.1098/rsbl.2021.0251

Jarrige, A., Kassis, A., Schmoll, T., Goubault, M.: **Recently mated males of a lek-mating insect intensify precopulatory mate guarding under male competition**, Animal Behaviour, 117. 21–34, 2016. https://doi.org/10.1016/J.ANBEHAV.2016.04.012

Jennions, M. D., Petrie, M.: **Why do females mate multiply? A review of the genetic benefits**, Biological Reviews of the Cambridge Philosophical Society, 75(1). 21–64, 2000. https://doi.org/10.1017/S0006323199005423

Jormalainen, V.: **Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict**, Quarterly Review of Biology, 73(3). 275–304, 1998. https://doi.org/10.1086/420306

Karlsson, B.: **Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly**, Ecology, 79(8). 2931–2940, 1998. https://doi.org/10.1890/0012-9658(1998)079[2931:NGRBAR]2.0.CO;2

Kawagoe, T., Suzuki, N., Matsumoto, K.: **Multiple mating reduces longevity of females of the windmill butterfly *Atrophaneura alcinous***, Ecological Entomology, 26(3). 258–262, 2001. https://doi.org/10.1046/j.1365-2311.2001.00326.x

Keller, L., Reeve, H. K.: **Why do females mate with multiple males? The sexually selected sperm hypothesis**, In: *Advances in the Study of Behaviour*, Academic Press, 1995.

King, B. H., Fischer, C. R.: **Males mate guard in absentia through extended effects of postcopulatory courtship in the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae)**, Journal of Insect Physiology, 51(12). 1340–1345, 2005. https://doi.org/10.1016/j.jinsphys.2005.08.004

Knox, T. T., Scott, M. P.: **Size, operational sex ratio, and mate-guarding success of the carrion beetle, *Necrophila americana***, Behavioral Ecology, 17(1). 88–96, 2006. https://doi.org/10.1093/BEHECO/ARJ004

Konvička, M., Duchoslav, M., Haraštová, M., Beneš, J., Foldynová, S., Jirků, M., Kuras, T.: **Habitat utilization and behaviour of adult *Parnassius mnemosyne* (Lepidoptera: Papilionidae) in the Litovelské Pomoraví, Czech Republic**, Nota Lepidopterologica, 24(1–2). 39–51, 2001.

Konvička, M., Kuras, T.: **Population structure, behaviour and selection of oviposition sites of an endangered butterfly, *Parnassius mnemosyne*, in Litovelske Pomoravi, Czech Republic**, Journal of Insect Conservation, 3(3). 211–223, 1999. https://doi.org/10.1023/A:1009641618795

Kvarnemo, C., Ahnesjö, I.: **Operational sex ratios and mating competition**, In: *Sex Ratios: Concepts and Research Methods*, I. C. W. Hardy (Ed.), Cambridge University Press, 2002. https://doi.org/10.1017/CBO9780511542053.019

Labine, P. A.: **The population biology of the butterfly, *Euphydryas editha*. IV. Sperm precedence - A preliminary report**, Evolution, 20(4). 580–586, 1966. https://doi.org/10.2307/2406592

Lenth, R. V.: **Least-squares means: the R package lsmeans**, Journal of Statistical Software, 69(1). 1–33, 2016. https://doi.org/10.18637/JSS.V069.I01

Mahoney, P. C., Tatarnic, N. J., O'Hanlon, J. C., Umbers, K. D. L.: **Mate guarding and male mate choice in the chameleon grasshopper *Kosciuscola tristis* (Orthoptera: Acrididae)**, Journal of Ethology, 35(2). 197–201, 2017. https://doi.org/10.1007/S10164-017-0509-9

Manning, J. T.: **Male discrimination and investment in *Asellus aquaticus* (L.) and *A. meridianus* Racovitsza (Crustacea: Isopoda)**, Behaviour, 55(1–2). 1–14, 1975. https://doi.org/10.1163/156853975X00380

Marshall, G. A. K.: **On the female pouch in *Acraea***, The Entomologist, 34. 73–75, 1901.

Marshall, L. D., McNeil, J. N.: **Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae)**, Functional Ecology, 3(5). 605–612, 1989. https://doi.org/10.2307/2389575

Martin, O. Y., Hosken, D. J.: **The evolution of reproductive isolation through sexual conflict**, Nature, 423(6943). 979–982, 2003. https://doi.org/10.1038/nature01752

Matsumoto, K.: **Mating patterns of a sphragis-bearing butterfly, *Luehdorfia japonica* Leech (Lepidoptera: Papilionidae), with descriptions of mating behavior**, Researches on Population Ecology, 29(1). 97–110, 1987. https://doi.org/10.1007/BF02515428

Matsumoto, K., Orr, A. G., Yago, M.: **The occurrence and function of the sphragis in the Zerynthiine genera *Zerynthia*, *Allancastria* and *Bhutanitis* (Lepidoptera: Papilionoidea: Papilionidae)**, Journal of Natural History, 52(21–22). 1351–1376, 2018. https://doi.org/10.1080/00222933.2018.1464228

Matsumoto, K., Suzuki, N.: **Effectiveness of the mating plug in *Atrophaneura alcinous* (Lepidoptera: Papilionidae)**, Behavioral Ecology and Sociobiology, 30(3–4). 157–163, 1992. https://doi.org/10.1007/BF00166698

Matsumoto, K., Suzuki, N.: **The nature of mating plugs and the probability of reinsemination in Japanese Papilionidae**, In: *Swallowtail Butterflies: Their Ecology and Evolutionary Biology*, J. M. Schriber, Y. Tsubaki, R. C. Lederhous (Eds.), Scientific Publishers, 1995.

Matter, S. F., Reed, B., Illerbrun, K., Doyle, A., McPike, S., Roland, J.: **Young love? Mating of *Parnassius smintheus* Doubleday (Papilionidae)**, Journal of the Lepidopterists' Society, 66(2). 111–113, 2012. https://doi.org/10.18473/lepi.v66i2.a4

McCorkle, D. V., Hammond, P. C.: **Observations on the biology of *Parnassius clodius* (Papilionidae) in the Pacific Northwest**, Journal of the Lepidopterists Society, 39(3). 156–162, 1985.

Meglécz, E., Nève, G., Pecsenye, K., Varga, Z.: **Genetic variations in space and time in *Parnassius mnemosyne* (L.) (Lepidoptera) populations in north-east Hungary: implications for conservation**, Biological Conservation, 89(3). 251–259, 1999. https://doi.org/10.1016/S0006-3207(99)00006-3

Meier, K., Kuusemets, V., Luig, J., Mander, Ü.: **Riparian buffer zones as elements of ecological networks: case study on *Parnassius mnemosyne* distribution in Estonia**, Ecological Engineering, 24(5). 531–537, 2005. https://doi.org/10.1016/j.ecoleng.2005.01.017

Mo, H.-ho, Jang, K. B., Park, J.-J., Lee, S.-E., Shin, K.-I., Lee, J.-H., Cho, K.: **Interactive effect of diet and temperature on instar numbers in *Spodoptera litura*, with reference to head capsule width and weight**, Journal of Asia-Pacific Entomology, 16(4). 521–525, 2013. https://doi.org/10.1016/J.ASPEN.2013.08.007

Niihara, S., Watanabe, M.: **Effects of saline intake until the second mating on the ejaculates and sperm number of male swallowtail butterfly, *Papilio xuthus* Linnaeus**, Japanese Journal of Applied Entomology and Zoology, 53(3). 91–96, 2009. https://doi.org/10.1303/jjaez.2009.91

Nilakhe, S. S.: **Longevity and fecundity of female boll weevils placed with varying numbers of males**, Annals of the Entomological Society of America, 70(5). 673–674, 1977. https://doi.org/10.1093/AESA/70.5.673

Oberhauser, K. S.: **Fecundity, lifespan and egg mass in butterflies: effects of male-derived nutrients and female size**, Functional Ecology, 11(2). 166–175, 1997. https://doi.org/10.1046/J.1365-2435.1997.00074.X

Okada, K., Katsuki, M., Sharma, M. D., Kiyose, K., Seko, T., Okada, Y., Wilson, A. J., Hosken, D. J.: **Natural selection increases female fitness by reversing the exaggeration of a male sexually selected trait**, Nature Communications, 12(3420). 1–10, 2021. https://doi.org/10.1038/s41467-021-23804-7

Orr, A. G.: **Mate conflict and the evolution of the sphragis in butterflies***,* PhD thesis, School of Australian Environmental Studies, Griffith University, 1988.

Orr, A. G.: **The evolution of the sphragis in the Papilionidae and other butterflies**, In: *Swallowtail Butterflies: Their Ecology and Evolutionary Biology*, J. M. Schriber, Y. Tsubaki, R. C. Lederhous (Eds.), Scientific Publishers, 1995.

Orr, A. G.: **The big greasy, *Cressida cressida* (Papilionidae)**, In: *Monographs on Australian Lepidoptera, Biology of Australian Butterflies*, R. L. Kitching, E. Scheermeyer, R. E. Jones, N. E. Pierce (Eds.), CSIRO Publishing, 1999.

Orr, A. G.: **The sphragis of *Heteronympha penelope* Waterhouse (Lepidoptera: Satyridae): its structure, formation and role in sperm guarding**, Journal of Natural History, 36(2). 185–196, 2002. https://doi.org/10.1080/00222930010022926

Orr, A. G., Rutowski, R. L.: **The function of the sphragis in *Cressida cressida* (Fab.) (Lepidoptera, Papilionidae): A visual deterrent to copulation attempts**, Journal of Natural History, 25(3). 703–710, 1991. https://doi.org/10.1080/00222939100770461

Parker, G. A.: **The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). IV. Epigamic recognition and competition between males for the possession of females**, Behaviour, 37(1–2). 113–139, 1970. https://doi.org/10.1163/156853970X00268

Parker, G. A.: **Sexual selection and sexual conflict**, In: *Sexual Selection and Reproductive Competition in Insects*, M. S. Blum, N. A. Blum (Eds.), Academic Press, 1979. https://doi.org/10.1016/b978-0-12-108750-0.50010-0

Parker, G. A.: **Mate quality and mating decisions**, In: *Mate Choice*, P. Bateson (Ed.), Cambridge University Press, 1983.

Parker, G. A.: **Sperm competition and the evolution of animal mating strategies**, In: *Sperm Competition and the Evolution of Animal Mating Strategies*, R. L. Smith (Ed.), Academic Press, 1984.

Parker, G. A.: **Sexual conflict over mating and fertilization: an overview**, Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1466). 235–259, 2006. https://doi.org/10.1098/rstb.2005.1785

Parker, G. A., Baker, R. R., Smith, V. G. F.: **The origin and evolution of gamete dimorphism and the male-female phenomenon**, Journal of Theoretical Biology, 36(3). 529–553, 1972. https://doi.org/10.1016/0022-5193(72)90007-0

Partridge, L., Hurst, L. D.: **Sex and conflict**, Science, 281(5385). 2003–2008, 1998. https://doi.org/10.1126/science.281.5385.2003

Pásztor, K., Kőrösi, Á., Gór, Á., Szigeti, V., Vajna, F., Kis, J.: **Phenotypic senescence in a natural insect population**, Ecology and Evolution, 12(e9668). 1–13, 2022. https://doi.org/10.1002/ECE3.9668

Pérez-Staples, D., Harmer, A. M. T., Taylor, P. W.: **Sperm storage and utilization in female Queensland fruit flies (*Bactrocera tryoni*)**, Physiological Entomology, 32(2). 127–135, 2007. https://doi.org/10.1111/j.1365-3032.2006.00554.x

Petersen, W.: **Über die sphragis und das spermatophragma der tagfaltergattung *Parnassius* (Lep.)**, Berliner Entomologische Zeitschrift, 5. 407–413, 1928. https://doi.org/10.1002/MMND.192919280506

Peterson, B. G., Carl, P.: *PerformanceAnalytics: econometric tools for performance and risk analysis,* 2020.

Pierre, J.: **Le sphragis chez les Acraeinae (Lepidoptera Nymphalidae) [The sphragis in the Acraeinae (Lepidoptera Nymphalidae)]**, Annales de La Société Entomologique de France, 21(4). 393–398, 1985.

Pinheiro, J., Bates, D.: *nlme: linear and nonlinear mixed effects models,* 2023.

Polak, M., Wolf, L. L., Starmer, W. T., Barker, J. S. F.: **Function of the mating plug in *Drosophila hibisci* Bock**, Behavioral Ecology and Sociobiology, 49(2). 196–205, 2001. https://doi.org/10.1007/s002650000281

Prenter, J., Elwood, R. W., Montgomery, W. I.: **Assessments and decisions in *Metellina segmentata* (Araneae: Metidae): evidence of a pheromone involved in mate guarding**, Behavioral Ecology and Sociobiology, 35(1). 39–43, 1994. https://doi.org/10.1007/BF00167058

Putter, H., Fiocco, M., Gekus, R. B.: **Tutorial in biostatistics: competing risks and multi-state models**, Statistics in Medicine, 26(11). 2389–2430, 2007. https://doi.org/10.1002/SIM.2712

R Core Team: **R: A language and environment for statistical computing**, In: *R Foundation for Statistical Computing,* 2023.

Roland, J., Keyghobadi, N., Fownes, S.: **Alpine *Parnassius* butterfly dispersal: effects of landscape and population size**, Ecology, 81(6). 1642–1653, 2000. https://doi.org/10.2307/177313

Ronkay, L.: **Nemzeti biodiverzitás monitorozó rendszer VII. Lepkék***,* Magyar Természettudományi Múzeum, 1997.

Rönn, J., Katvala, M., Arnqvist, G.: **Coevolution between harmful male genitalia and female resistance in seed beetles**, Proceedings of the National Academy of Sciences of the United States of America, 104(26). 10921–10925, 2007. https://doi.org/10.1073/pnas.0701170104

Rowe, L.: **Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration**, Animal Behaviour, 44(2). 189–202, 1992. https://doi.org/10.1016/0003-3472(92)90025-5

Saigusa, T.: **A phylogeny of the genus *Luehdorfia* (I)**, Konchu-to-Shizen, 8. 5–18, 1973.

Sakaluk, S. K.: **Post-copulatory mate guarding in decorated crickets**, Animal Behaviour, 41(2). 207–216, 1991. https://doi.org/10.1016/S0003-3472(05)80472-5

Sauter, A., Brown, M. J. F., Baer, B., Schmid-Hempel, P.: **Males of social insects can prevent queens from multiple mating**, Proceedings of the Royal Society of London. Series B: Biological Sciences, 268(1475). 1449–1454, 2001. https://doi.org/10.1098/rspb.2001.1680

Schmidt, C. D., Blume, R. R.: **Laboratory-reared horn flies: relationships between width of head and weight of pupa in both sexes and between these measurements and number of ovarioles in females**, Annals of the Entomological Society of America, 66(6). 1307–1308, 1973. https://doi.org/10.1093/AESA/66.6.1307

Schneider, C. A., Rasband, W. S., Eliceiri, K. W.: **NIH Image to ImageJ: 25 years of image analysis**, Nature Methods, 9(7). 671–675, 2012. https://doi.org/10.1038/nmeth.2089

Schöfl, G., Taborsky, M.: **Prolonged tandem formation in firebugs (*Pyrrhocoris apterus*) serves mate-guarding**, Behavioral Ecology and Sociobiology, 52(5). 426–433, 2002. https://doi.org/10.1007/S00265-002-0524-9

Schröder, T.: **Precopulatory mate guarding and mating behaviour in the rotifer *Epiphanes senta* (Monogononta: Rotifera)**, Proceedings of the Royal Society. Series B: Biological Sciences, 270(1527). 1965–1970, 2003. https://doi.org/10.1098/rspb.2003.2466

Scoble, M. J.: **The Lepidoptera. Form, function and diversity**, In: *The Lepidoptera. Form, function and diversity,* Oxford University Press, 1992.

Seed, C. E., Tomkins, J. L.: **Positive size–speed relationships in gametes and vegetative cells of *Chlamydomonas reinhardtii*; implications for the evolution of sperm**, Evolution, 72(3). 440–452, 2018. https://doi.org/10.1111/evo.13427

Shuster, S. M.: **Sexual selection in the socorro isopod, *Thermosphaeroma thermophilum* (Cole) (Crustacea: Peracarida)**, Animal Behaviour, 29(3). 698–707, 1981. https://doi.org/10.1016/S0003-3472(81)80004-8

Sibley, P. K., Ankley, G. T., Benoit, D. A.: **Factors affecting reproduction and the importance of adult size on reproductive output of the midge *Chironomus tentans***, Environmental Toxicology and Chemistry, 20(6). 1296–1303, 2001. https://doi.org/10.1002/etc.5620200618

Simmons, L. W.: **The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses**, Journal of Evolutionary Biology, 14(4). 585–594, 2001. https://doi.org/10.1046/j.1420-9101.2001.00309.x

Simmons, L. W.: **Sperm Competition and Its Evolutionary Consequences in the Insects**, In: *Sperm Competition and Its Evolutionary Consequences in the Insects,* Princeton University Press, 2002.

Sims, S. R.: **Aspects of mating frequency and reproductive maturity in *Papilio zelicaon***, American Midland Naturalist, 102(1). 36–50, 1979. https://doi.org/10.2307/2425064

Stjernholm, F., Karlsson, B.: **Nuptial gifts and the use of body resources for reproduction in the green–veined white butterfly *Pieris napi***, Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1445). 807–811, 2000. https://doi.org/10.1098/rspb.2000.1075

Stjernholm, F., Karlsson, B., Boggs, C. L.: **Age-related changes in thoracic mass: possible reallocation of resources to reproduction in butterflies**, Biological Journal of the Linnean Society, 86(3). 363–380, 2005. https://doi.org/10.1111/J.1095-8312.2005.00542.X

Stockley, P., Franco, C., Claydon, A. J., Davidson, A., Hammond, D. E., Brownridge, P. J., Hurst, J. L., Beynon, R. J.: **Revealing mechanisms of mating plug function under sexual selection**, Proceedings of the National Academy of Sciences of the United States of America, 117(44). 27465–27473, 2020. https://doi.org/10.1073/pnas.1920526117

Stoffer, D., Poison, N.: *astsa: applied statistical time series analysis,* 2023.

Stutt, A. D., Siva-Jothy, M. T.: **Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius***, Proceedings of the National Academy of Sciences of the United States of America, 98(10). 5683–5687, 2001. https://doi.org/10.1073/pnas.101440698

Summers, S. L., Kawahara, A. Y., Carvalho, A. P. S.: **Effects of the sphragis on male and female genitalia in *Acraea* (Nymphalidae) butterflies**, UF Journal of Undergraduate Research, 22. 2020. https://doi.org/10.32473/ufjur.v22i0.121244

Sutter, A., Lindholm, A. K.: **The copulatory plug delays ejaculation by rival males and affects sperm competition outcome in house mice**, Journal of Evolutionary Biology, 29(8). 1617–1630, 2016. https://doi.org/10.1111/jeb.12898

Sydsæter, K., Hammond, P., Strom, A., Carvajal, A.: **Essential Mathematics for Economic Analysis***,* Pearson Education, 2016.

Szigeti, V., Kőrösi, Á., Harnos, A., Kis, J.: **Temporal changes in floral resource availability and flower visitation in a butterfly**, Arthropod-Plant Interactions, 12(2). 177–189, 2018. https://doi.org/10.1007/s11829-017-9585-6

Szigeti, V., Kőrösi, Á., Harnos, A., Kis, J.: **Lifelong foraging and individual specialisation are influenced by temporal changes of resource availability**, Oikos, 128(5). 649–658, 2019. https://doi.org/10.1111/OIK.05400

Szigeti, V., Kőrösi, Á., Harnos, A., Nagy, J., Kis, J.: **Comparing two methods for estimating floral resource availability for insect pollinators in semi-natural habitats**, Annales de La Societe Entomologique de France, 52(5). 289–299, 2016. https://doi.org/10.1080/00379271.2016.1261003

Szigeti, V., Vajna, F., Kőrösi, Á., Kis, J.: **Are all butterflies equal? Population-wise proboscis length variation predicts flower choice in a butterfly**, Animal Behaviour, 163. 135–143, 2020. https://doi.org/10.1016/J.ANBEHAV.2020.03.008

Takeshita, F., Henmi, Y.: **The effects of body size, ownership and sex-ratio on the precopulatory mate guarding of *Caprella penantis* (Crustacea: Amphipoda)**, Journal of the Marine Biological Association of the United Kingdom, 90(2). 275–279, 2010. https://doi.org/10.1017/S0025315409990671

Teder, T., Kaasik, A., Taits, K., Tammaru, T.: **Why do males emerge before females? Sexual size dimorphism drives sexual bimaturism in insects**, Biological Reviews, 96(6). 2461–2475, 2021. https://doi.org/10.1111/BRV.12762

Tewksbury, J. J.: **Fruits, frugivores and the evolutionary arms race**, New Phytologist, 156(2). 137–139, 2002. https://doi.org/10.1046/j.1469-8137.2002.00522.x

Therneau, T.: *survival: package for survival analysis in R,* 2023.

Therneau, T. M., Grambsch, P. M.: **Modeling Survival Data: Extending the Cox Model***,* Springer, 2000. https://doi.org/10.1007/978-1-4757-3294-8_3

Thompson, D. J., Manning, J. T.: **Mate selection by *Asellus* (Crustacea: Isopoda)**, Behaviour, 78(3–4). 178–186, 1981. https://doi.org/10.1163/156853981X00310

Thornhill, R., Alcock, J.: **The Evolution of Insect Mating Systems**, In: *The Evolution of Insect Mating Systems,* Harvard University Press, 1983. https://doi.org/10.4159/HARVARD.9780674433960

Timmermeyer, N., Gerlach, T., Guempel, C., Knoche, J., Pfann, J. F., Schliessmann, D., Michiels, N. K.: **The function of copulatory plugs in *Caenorhabditis remanei*: hints for female benefits**, Frontiers in Zoology, 7(28). 1–7, 2010. https://doi.org/10.1186/1742-9994-7-28

Togashi, T., Bartelt, J. L., Yoshimura, J., Tainaka, K., Cox, P. A.: **Evolutionary trajectories explain the diversified evolution of isogamy and anisogamy in marine green algae**, Proceedings of the National Academy of Sciences, 109(34). 13692–13697, 2012. https://doi.org/10.1073/pnas.1203495109

Togashi, T., Horinouchi, Y., Sasaki, H., Yoshimura, J.: **Evidence for equal size cell divisions during gametogenesis in a marine green alga *Monostroma angicava***, Scientific Reports, 5(1). 1–9, 2015. https://doi.org/10.1038/srep13672

Tregenza, T., Wedell, N.: **Polyandrous females avoid costs of inbreeding**, Nature, 415(6867). 71–73, 2002. https://doi.org/10.1038/415071a

Tregenza, T., Wedell, N., Chapman, T.: **Introduction. Sexual conflict: a new paradigm?**, Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1466). 229–234, 2006. https://doi.org/10.1098/rstb.2005.1796

van Swaay, C., Cuttelod, A., Collins, S., Maes, D., López Munguira, M., Šašic, M., Settele, J., Verovnik, R., Verstrael, T., Warren, M., Wiemers, M., Wynhoff, I.: **European Red List of butterflies***,* Publications Office of the European Communities, 2010.

Vande Velde, L., Schtickzelle, N., Van Dyck, H.: **Effect of larval food stress on male adult behaviour, morphology and reproductive investment in the butterfly *Pararge aegeria***, Evolutionary Ecology, 27(1). 221–234, 2013. https://doi.org/10.1007/s10682-012-9580-4

Vlašánek, P., Hauck, D., Konvička, M.: **Adult sex ratio in the *Parnassius mnemosyne* butterfly: effects of survival, migration, and weather**, Israel Journal of Ecology and Evolution, 55(3). 233–252, 2009. https://doi.org/10.1560/IJEE.55.3.233

Vlašánek, P., Konvička, M.: **Sphragis in *Parnassius mnemosyne* (Lepidoptera: Papilionidae): male-derived insemination plugs loose efficiency with progress of female flight**, Biologia, 64(6). 1206–1211, 2009. https://doi.org/10.2478/s11756-009-0207-3

Vojnits, A. M., Ács, E.: **Biology and behaviour of a Hungarian population of *Parnassius mnemosyne*: (Linnaeus, 1758)**, Oedippus, 17. 1–24, 2000.

Watanabe, M.: **Sperm Competition in Butterflies***,* Springer Japan, 2016. https://doi.org/10.1007/978-4-431-55945-0

Weir, L. K., Grant, J. W. A., Hutchings, J. A.: **The influence of operational sex ratio on the intensity of competition for mates**, The American Naturalist, 177(2). 167–176, 2011. https://doi.org/10.1086/657918

Weiss, J. C.: **The Parnassiinae of the World***,* (Vol. 3) Hillside Books, 1999.

Wickham, H.: **ggplot2: Elegant Graphics for Data Analysis***,* Springer, 2016. https://doi.org/10.1007/978-3-319-24277-4

Wiklund, C.: **Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants**, Oecologia, 63(1). 23–29, 1984. https://doi.org/10.1007/bf00379780

Xu, J., Wang, Q.: **Male moths undertake both pre- and in-copulation mate choice based on female age and weight**, Behavioral Ecology and Sociobiology, 63(6). 801–808, 2009. https://doi.org/10.1007/S00265-009-0713-X

Zheng, B., Wang, Y., Xia, C., Huang, D., Cao, Y., Hao, J., Zhu, C.: **The complete mitochondrial genome of *Parnassius actius* (Lepidoptera: Papilionidae: Parnassinae) with the related phylogenetic analysis**, Zoological Systematics, 43(1). 1–17, 2018. https://doi.org/10.11865/ZS.201801

Zorkóczy, O. K.: **Az időjárás, a testméret és a túlélés kapcsolata kis Apolló-lepke imágóknál [Association of adult survival with body size and weather in Clouded Apollo butterflies]***,* MSc thesis in Hungarian, University of Veterinary Medicine Budapest, 2020.

9. Contributions

Ádám Gór (author): Field work – data collection (Equal), Conceptualization (Supporting), Data curation (Equal), Image and video post-processing (Lead), Formal analysis (Lead), Investigation (Supporting), Methodology (Supporting), Visualisation (Equal), Writing – original draft (Lead), Writing – review editing (Lead)

János Kis (supervisor): Field work – data collection (Equal), Conceptualization (Lead), Investigation (Lead), Methodology (Lead), Project administration (Lead), Supervision (Equal), Visualization (Equal), Writing – original draft (Supporting), Writing – review editing (Supporting)

Zsolt Lang (co-supervisor): Formal analysis (Supporting), Supervision (Equal), Writing – original draft (Supporting), Writing – review editing (Supporting)

Kata Pásztor (co-author in articles): Field work – data collection (Equal), Data curation (Equal), Investigation (Supporting)

Viktor Szigeti (co-author in articles): Field work – data collection (Equal), Conceptualization (Supporting), Investigation (Supporting), Methodology (Supporting)

Flóra Vajna (co-author in articles): Field work – data collection (Equal), Data curation (Equal), Investigation (Supporting)

10. The author's publications

10.1. Full-text publications in peer-reviewed journals with an impact factor assigned

1. Gór, Á., Fónagy, A., Pásztor, K., Szigeti, V., Lang, Z., Kis, J. **Facultative male investment in prolonged mate-guarding in a butterfly.** Behaviour, 160(6), 515-557, 2023. https://doi.org/10.1163/1568539X-bja10219, (IF$_{2022-23}$: 1.672)

2. Gór, Á., Lang, Z., Pásztor, K., Szigeti, V., Vajna, F., Kis, J. **Mate-guarding success depends on male investment in a butterfly.** Ecology and Evolution, 13, e10533, 2023. https://doi.org/10.1002/ece3.10533, (IF$_{2022-23}$: 3.167)

10.2. Full-text publication in a peer-reviewed journal with no impact factor assigned

1. Gór, Á., Fónagy, A., Pásztor, K., Szigeti, V., Lang, Z., Kis, J. **Változó hím befektetés a nőstény további párosodását akadályozó képletekbe kis apollólepkénél (*Parnassius mnemosyne* (LINNAEUS, 1758)) [Variable male investment in devices impeding female remating in the Clouded Apollo butterfly (*Parnassius mnemosyne* (LINNAEUS, 1758))].** Állattani Közlemények 108(1-2), 2023. http://dx.doi.org/10.20331/AllKoz.2023.108.1-2.3

10.3. Oral presentations at international and Hungarian conferences

1. Gór, Á., Szigeti, V., Kis, J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási stratégiái.** Magyar Etológus Konferencia. Debrecen, 2016.

2. Gór, Á., Szigeti, V., Kis, J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási stratégiái.** Akadémiai Beszámolók, Budapest, 2017.

3. Gór, Á. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási stratégiái.** XXXIII. Országos Tudományos Diákköri Konferencia, Debrecen, 2017.
4. Gór, Á., Csintalan, Z., Szigeti, V., Kis, J. **A kis Apolló-lepkék (*Parnassius mnemosyne*) virágszín preferenciája lepke látás alapján.** Magyar Etológus Konferencia. Dobogókő, 2017.
5. Gór, Á. **Erényövek és párzási dugók cserélődési rátája kis Apolló-lepkéknél (*Parnassius mnemosyne*).** XXXIV. Országos Tudományos Diákköri Konferencia, Budapest, 2019.
6. Gór, Á., Kis, J. **Erényövek és párzási dugók cserélődési rátája kis Apolló-lepkéknél (*Parnassius mnemosyne*).** Akadémiai Beszámolók, Budapest, 2019.
7. Gór, Á., Lang, Z., Vajna, F., Szigeti, V., Kis, J. **Prolonged mate-guarding and male investment dynamics in Clouded Apollo butterflies, *Parnassius mnemosyne* (Papilionidae: Parnassiinae).** Reproductive strategies from genes to societies - Frontiers in animal and plant reproduction research. Debrecen, 2019.
8. Gór, Á., Lang, Z., Vajna, F., Szigeti, V., Kis, J. **Erényövek és párzási dugók cserélődési rátája kis Apolló-lepkéknél (*Parnassius mnemosyne*).** Magyar Etológus Konferencia. Mátrafüred, 2019.
9. Pásztor, K., Kőrösi, Á., Gór, Á., Kis, J. **Multilevel variation in body mass and thorax width in a natural butterfly population.** XXII. European Congress of Lepidopterology. Laulasmaa, Estonia, 2022.
10. Pásztor, K., Kőrösi, Á., Gór, Á., Kis, J. **How weather and body size affect survival and detectability in a natural butterfly population.** 8th Student Conference on Conservation Science Europe. Balatonvilágos, 2023.
11. Kis, J., Gór, Á., Lang, Z., Fónagy, A., Pásztor, K., Szigeti, V., Vajna, F. **Guarding success depends on facultative male investment in mate-guarding in Clouded Apollo butterflies.** Magyar Etológus Konferencia. Debrecen, 2023.

10.4. Poster presentations at international and Hungarian conferences

1. Pásztor, K., Kőrösi, Á., Gór, Á., Kis, J. **Change of body mass and thorax width with age in a natural butterfly population.** 2021 Annual Meeting of the Lepidopterists' Society. Online conference, 2021.

2. Gór, Á., Pásztor, K., Szabó, K., Kis, J. **Does sexual dimorphism in wing colouration relate to sphragis shape in Apollo (*Parnassius*) butterflies?** XXII. European Congress of Lepidopterology. Laulasmaa, Estonia, 2022.

3. Pásztor, K., Kőrösi, Á., Gór, Á., Kis, J. **Change of body mass and thorax width with age in a natural butterfly population.** 7th Student Conference on Conservation Science Europe. Balatonvilágos, 2022.

4. Pásztor, K., Kőrösi, Á., Gór, Á., Kis, J. **How weather and body size affect survival and detectability in a natural butterfly population.** International Conference on the Biology of Butterflies. Prague, Czech Republic, 2023.

10.5. Publications not related to the topic of the thesis

1. Gór, Á., Szigeti, V., Kis, J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási stratégiái.** Magyar Állatorvosok Lapja 139(4), 249, 2017.

2. Gergely, P., Gór, Á., Hudák, T., Ilonczai, Z., Szombathelyi, E. Nappali lepkéink – **Határozó terepre és természetfotókhoz / A Field Guide to the Butterflies of Hungary.** Kitaibel Kiadó, pp. 264, 2017.

3. Gór, Á. **Lepkefaunisztikai kutatások Biatorbágyon és környékén (Lepidoptera). Lepidoptera survey in Biatorbágy (Hungary) and its surrounding areas.** eActa Naturalia Pannonica 16: 55–70, 2018. http://dx.doi.org/10.24369/eANP.2018.16.55

4. Gergely, P., Gór, Á., Hudák, T., Ilonczai, Z., Szombathelyi, E.: **Nappali lepkéink – Határozó terepre és természetfotókhoz (Második, javított kiadás) – A Field Guide to the Butterflies of Hungary (Second, corrected edition).** Kitaibel Kiadó, pp. 264, 2018.

5. Maes, D., Verovnik, R., Wiemers, M., Brosens, D., Beshkov, S., Bonelli, S., Buszko, J., Cantú-Salazar, L., Cassar, L.-F., Collins, S., Dincă, V., Djuric, M., Dušej, G., Elven, H., Franeta, F., Garcia-Pereira, P., Geryak, Y., Goffart, P., Gór, Á. et al. **Integrating national Red Lists for prioritising conservation actions for European butterflies.** Journal of Insect Conservation 23, 301–330, 2019. https://doi.org/10.1007/s10841-019-00127-z

6. Juhász, E., Gór, Á., Bali, D., Kerékgyártó, F., Katona, G., Végvári, Z. **Long-term temporal patterns in flight activities of a migrant diurnal butterfly.** Insect Science 28, 839-849, 2021. https://doi.org/10.1111/1744-7917.12815

7. Juhász, E., Németh, Z., Gór, Á., Végvári, Z. **Multilevel climatic responses in migratory insects.** Ecological Entomology 48(6), 755–764, 2023. https://doi.org/10.1111/een.13270

8. Pásztor, K., Kőrösi, Á., Gór, Á., Szigeti, V., Vajna, F., Kis, J. **Phenotypic senescence in a natural insect population.** Ecology and Evolution, 12, e9668, 2022. https://doi.org/10.1002/ece3.9668

11. Appendices

Appendix 1. The table shows the results of all multistate submodels including those omitted from the Results. These were omitted due to their mostly non-significant, uncertain and non-interpretable effects.

Explanatory variable	Number of females	Number of transitions	Transition (dependent variables)	RR	SE	*p*	CI
Short vs. long shields (binary)	348	1205	Shield to small CAP	27.56	0.65	< 0.001	5.83, 130.40
			Shield to disappeared	1.08	1.49	0.554	0.85, 1.36
			Shield to shield	25.42	0.85	0.003	2.96, 218.60
Thorax width to head width ratio (continuous)	492	1190	No CAP to small CAP	0.66	1.00	0.665	0.10, 4.30
			No CAP to shield	3.70	0.72	0.012	1.34, 10.23
			No CAP to disappeared	0.16	1.64	0.199	0.01, 2.59
			Small CAP to no CAP	8.79×10^{3}	6.06	0.050	1.00, 7.72×10^{7}
			Small CAP to shield	503.70	2.32	0.002	9.06, 2.80×10^{4}
			Small CAP to disappeared	7.16×10^{-4}	1.21	< 0.001	7.56×10^{-5}, 6.79×10^{-3}
			Small CAP to small CAP	1.77	1.28	0.515	0.32, 9.81
			Shield to small CAP	4.23	3.52	0.710	2.13×10^{-3}, 8.40×10^{3}
			Shield to disappeared	0.05	0.73	< 0.001	0.01, 0.18
			Shield to shield	1.11	4.48	0.976	1.10×10^{-3}, 1.12×10^{3}
Head width (continuous)			No CAP to small CAP	0.22	0.87	0.072	0.06, 1.13
			No CAP to shield	3.99	0.56	< 0.001	1.89, 8.42
			No CAP to disappeared	9.68×10^{-3}	1.60	< 0.001	6.56×10^{-4}, 1.43×10^{-1}
			Small CAP to no CAP	12.04	4.46	0.539	4.28×10^{-3}, 3.39×10^{4}
			Small CAP to shield	16.75	1.97	0.133	0.42, 662.50
			Small CAP to disappeared	0.15	1.14	0.037	0.02, 0.89
			Small CAP to small CAP	1.54	1.21	0.637	0.26, 9.34
			Shield to small CAP	0.55	2.44	0.808	4.24×10^{-3}, 70.58
			Shield to disappeared	0.35	0.56	0.026	0.14, 0.88
			Shield to shield	0.82	3.55	0.948	2.53×10^{-3}, 2.69×10^{2}
Male-biased vs. female-biased ASR (binary)		1206	No CAP to small CAP	0.49	0.20	< 0.001	0.33, 0.72
			No CAP to shield	3.67	0.26	< 0.001	2.21, 6.09
			No CAP to disappeared	0.38	0.32	0.003	0.21, 0.72
			Small CAP to no CAP	3.24	1.08	0.278	0.39, 27.08

			Small CAP to shield	7.04	0.61	0.001	2.12, 23.41
			Small CAP to disappeared	0.31	0.22	< 0.001	0.20, 0.47
			Small CAP to small CAP	1.07	0.22	0.777	0.69, 1.64
			Shield to small CAP	1.07	1.04	0.947	0.14, 8.23
			Shield to disappeared	0.41	0.18	< 0.001	0.29, 0.58
			Shield to shield	1.43×10^6	1.48×10^3	0.992	0, Infinity
Body mass (continuous)	441	1079	No CAP to small CAP	6.87×10^{-4}	2.49	0.002	7.21×10^{-6}, 6.54×10^{-2}
			No CAP to shield	2.26	1.57	0.461	0.27, 20.00
			No CAP to disappeared	0.01	4.02	0.253	1.07×10^{-5}, 20.42
			Small CAP to no CAP	8.87×10^9	11.03	0.045	1.61, 4.90×10^{19}
			Small CAP to shield	1.58×10^3	5.17	0.132	0.11, 2.26×10^7
			Small CAP to disappeared	4.55×10^{-5}	3.42	0.003	5.64×10^{-8}, 3.67×10^{-2}
			Small CAP to small CAP	0.38	3.39	0.699	2.73×10^{-3}, 52.34
			Shield to small CAP	7.60×10^{-3}	8.01	0.548	9.26×10^{-10}, 6.23×10^4
			Shield to disappeared	8.97×10^{-3}	1.72	0.001	5.03×10^{-4}, 0.16
			Shield to shield	6.03×10^4	10.13	0.125	0.05, 7.85×10^{10}
Wing length (continuous)	479	1182	No CAP to small CAP	0.96	0.07	0.545	0.85, 1.09
			No CAP to shield	1.08	0.04	0.067	0.99, 1.18
			No CAP to disappeared	0.78	0.11	0.029	0.63, 0.98
			Small CAP to no CAP	1.36	0.26	0.246	0.81, 2.27
			Small CAP to shield	0.85	0.14	0.218	0.65, 1.10
			Small CAP to disappeared	0.91	0.09	0.273	0.77, 1.08
			Small CAP to small CAP	1.05	0.08	0.547	0.98, 1.24
			Shield to small CAP	1.03	0.20	0.872	0.70, 1.53
			Shield to disappeared	0.97	0.04	0.423	0.98, 1.05
			Shield to shield	1.06	0.30	0.844	0.59, 1.92
Proboscis length (continuous)	443	1105	No CAP to small CAP	0.84	0.14	0.188	0.64, 1.09
			No CAP to shield	1.25	0.10	0.032	1.02, 1.52
			No CAP to disappeared	0.66	0.20	0.037	0.45, 0.97
			Small CAP to no CAP	2.72	0.75	0.180	0.63, 11.70
			Small CAP to shield	0.95	0.31	0.875	0.51, 1.76
			Small CAP to disappeared	0.73	0.19	0.100	0.50, 1.06
			Small CAP to small CAP	0.99	0.20	0.965	0.67, 1.46
			Shield to small CAP	1.14	0.47	0.778	0.45, 2.88
			Shield to disappeared	0.87	0.11	0.202	0.70, 1.08
			Shield to shield	4.02	0.77	0.072	0.88, 18.27
Starting from no CAP	492	499	No CAP to shield	2.38	0.10	< 0.001	1.94, 2.91
			No CAP to shield * Time	1.38×10^{-3}	426.85	0.988	0, Infinity
			No CAP to disappeared	0.36	0.17	< 0.001	0.26, 0.50
			No CAP to disappeared * Time	1.15	0.09	0.127	0.96, 1.37
Starting from small CAP	145	247	Small CAP to no CAP	0.54	0.73	0.395	0.13, 2.25
			Small CAP to no CAP * Time	0.58	0.46	0.239	0.23, 1.44

			Small CAP to disappeared	1.77	0.24	0.018	1.11, 2.85
			Small CAP to disappeared * Time	1.16	0.04	< 0.001	1.08, 1.25
			Small CAP to small CAP	2.98	0.24	< 0.001	1.86, 4.77
			Small CAP to small CAP * Time	1.08	0.04	0.049	1.00, 1.17
Starting from shield	348	356	Shield to disappeared	21.43	0.26	< 0.001	12.84, 35.76
			Shield to disappeared * Time	0.99	0.03	0.581	0.93, 1.04
			Shield to shield	0.82	0.66	0.763	0.22, 3.01
			Shield to shield * Time	0.75	0.38	0.447	0.36, 1.57

Appendix 2. The table shows ANOVA models on the relationships of shield sizes and years. Note the insignificant differences between the models with all sphragides (upper three models) and the models excluding females that bore multiple shields during their life (lower three models). This supports not using mixed models.

Models with all sphragides (n = 356)	**Estimate**	**SE**	***p***
Shield length ~ year	0.062	0.047	0.191
Shield height ~ year	0.080	0.014	< 0.001
Shield width ~ year	-0.043	0.008	< 0.001
Truncated models (n = 340)			
Shield length ~ year	0.047	0.048	0.326
Shield height ~ year	0.074	0.014	< 0.001
Shield width ~ year	-0.042	0.009	< 0.001

12. Acknowledgements

I would like to thank and express my gratitude towards my supervisors, János Kis and Zsolt Lang, who helped and taught me a lot throughout the years. I am also grateful to my other co-authors, Viktor Szigeti, Flóra Vajna, Kata Pásztor and Adrien Fónagy, for their work with the manuscripts. Many students of the University of Veterinary Medicine Budapest collected the enormous dataset, creating the basis of this thesis. I am really grateful for their work and devotion. I am thankful for the drawings made by Zsuzsa Benedicty. László Peregovits, László Nagy Z. and Kornél Mauer provided logistic help. Viktor Kis allowed us to conduct field work on his land. I thank Kata Pásztor for reviewing the data and Dorottya Somlay for the head width data. I also thank all the reviewers and editors – Erzsébet Hornung, Gábor Bakonyi, Marc Jarmuszewski, Paul C. H. Albers, Chris Foote, Will Allen and four anonymous reviewers – for their constructive critiques on the three published articles. I am beyond grateful to my family and friends for their constant love, never-ending support and encouragement.