Summary

Populations respond to environmental changes through biological adaptations. Specialists, which rely heavily on specific ecological niches or interaction partners, face a rapid decline in their populations due to misadaptation to changing conditions when they are unable to adapt quickly enough. Thus, host-parasite systems are particularly vulnerable to extinction, due to parasite host dependency and low resilience to replace lost interactions. Reintroductions serve as a conservation tool to restore species loss and ecological processes after extinction. Additionally, successful reintroductions offer insights into evolutionary changes in new habitats, as a tool to learn whether and how organisms can deal with new environmental conditions. Despite the complexity of butterfly reintroductions, it has been one of the more popular taxa being reintroduced in the last decades, notably successful with *Phengaris* species reintroduced in the UK and Netherlands. Phengaris species, serving as biodiversity indicators, exhibit a specialized lifecycle as social parasites of ants, with adaptations facilitating integration within host colonies. Their lifecycle complexities underscore the importance of considering both the parasite and host species in conservation strategies. They rely on specific host plants and Myrmica ants. Mimicking chemical and vibroacoustic signals of ants, they infiltrate and integrate into the host colonies. Myrmica species differ in cuticular hydrocarbon profiles, which is the main nestmate recognition mechanism. Thus, Phengaris caterpillars need to mimic specific host ant chemical profiles to deceive ant workers of their Myrmica host species and facilitate adoption into the colony. Geographical variations in host specificity demonstrate a mosaic of coevolution, highlighting the spatially diverse nature of species interactions and adaptations. While evidence supports this pattern in cuckoo species, such as *Phengaris alcon*, it remains unknown for the most generalist predatory species, namely Phengaris teleius.

After the extinction of P. teleius in the Netherlands in 1976, a successful reintroduction occurred in 1990, involving the translocation of 86 butterflies from Poland to the Moerputten nature reserve in the Netherlands. The reintroduction effort resulted in the establishment of a metapopulation with thousands of individuals. Three decades later, this study aimed to assess potential changes in adult butterflies and caterpillars between the source and reintroduced metapopulations. The study encompasses chemical, vibroacoustic, behavioral analysis in caterpillars and ants; and morphological and genetic analysis in adult butterflies. In addition, the morphological changes in adult butterfly hindwings were evaluated spatially and temporarily by integrating data from current butterflies with historical data of individuals from the source and reintroduced metapopulations. The study provides insights into ongoing coevolution processes, adaptations to new conditions, and genetic impacts of reintroduction. We hypothesized that chemical profiles and vibroacoustic signals in caterpillars differ between metapopulations, resembling those of their sympatric Myrmica scabrinodis host ants. We also predicted more successful adoption and increased survival for caterpillars exposed to local host ants. Biotic and abiotic conditions likely influenced morphological traits differently between metapopulations, with the reintroduced metapopulation possibly exhibiting lower genetic variability. Moreover, metapopulation connectivity likely impacts selection pressure on butterfly morphology and dispersal. Additionally, factors like sexual selection, predation, and developmental stress may affect the hindwing spot pattern differently between metapopulations.

Our findings indicate that the reintroduced caterpillars differ in their chemical and vibroacoustic signals compared to their source metapopulation after 30 generations since the reintroduction.

Notably, the reintroduced metapopulation emitted vibroacoustic signals more akin to those of their sympatric ant hosts, suggesting potential for local adaptation. However, our analysis did not uncover any evidence of improved performance in chemical mimicry. The adult butterflies also present differences among metapopulations. The Polish butterflies from the current source metapopulation exhibited greater body weight and thorax size compared to the reintroduced ones. They also had the largest hindwings among all studied metapopulations (current and historical). The wing shape and spot pattern variation also differed between metapopulations. Metapopulation connectivity changed over time, decreasing slowly in Poland, but sharply increasing in the Netherlands after habitat restoration. Moreover, the genetic analysis revealed differences in allelic richness, indicating a founder effect and bottleneck in the reintroduced metapopulation, with clear genetic structure differentiation among metapopulations and lower effective population size in the reintroduced metapopulation compared to the source one.

The study highlights the adaptability of *P. teleius* across its life stages, showcasing the capacity of the caterpillars for coevolution and adaptation to new ant host metapopulations. Furthermore, morphological changes in adult butterflies were observed in response to environmental pressures. Moreover, as a consequence of the reintroduction process the reintroduced metapopulation exhibited a distinct genetic structure and showed resilience to genetic variability loss, facilitating successful colonization and increasing the metapopulation size. Evidence suggests that *P. teleius* is a promising candidate for reintroduction efforts capable of thriving and adapting in newly reintroduced habitats.