Summary

An important process contributing to evolutionary changes is trait loss. This field of research has, however, received little attention and its importance is typically overlooked. A trait can be considered lost when phenotypic expression is silenced under certain environmental conditions that warranted trait expression in the ancestral lineage. Trait loss can occur when a trait remains unused or when selection acts against it. The loss of traits can be accelerated when the environment compensates for a phenotypic function, in which a resource or function is provided by the environment, leaving the trait in the receiving organism prone to phenotypic degradation. Environmental compensation of traits can be realized in systems when the diet facilitates an essential resource or when an interacting partner provides a certain phenotypic function. Ecological interactions with the biotic environment are thus expected to play a key role in evolutionary trait dynamics.

Particularly important cases of trait loss pertain to traits that involve the loss of certain nutrient metabolic functions. Nutrition plays a key role in determining fitness through its inherent link to survival, growth and reproduction. An example in which an indispensable trait has been lost was found in parasitoids that lack lipid synthesis in their adult life-stage. These insects feed and develop on other arthropods during their larval stages, yet they are free-living as adults to search for new hosts. Lipogenesis has been considered a highly conserved pathway that is uniformly adopted by all animals to synthesize fatty acids used in the formation of triglycerides to store energy. Nutrient storage is particularly important when an animal is faced with unfavourable environmental conditions, in which food might not be available to meet direct energetic demands. Co-evolution between parasitoid and host was expected to fuel the loss of lipogenesis, since valuable resources in terms of lipid reserves are provided by the host during development. The parasitoid readily carries over these nutrients during development, compensating for this trait at the phenotypic level and rendering pathways underlying lipid synthesis prone to degradation. The first aspect of this hypothesis relates to the link between this metabolic aberration in larvae and adults: lack of lipogenesis is expected to be favoured in larvae that directly benefit from the host’s lipid supply, yet adults are constrained by the level of teneral lipid levels obtained from the host. The second aspect involves the ability of parasitoids to alter physiological processes in their
hosts, in which nutrient resources, including lipids, are increased through interference by the parasitoid. Host manipulation is thus expected to play an important role in the evolutionary loss of lipogenesis, because sufficient nutrient levels are required to sustain metabolic functions in the adult life-stage.

Three pertinent questions regarding the evolutionary loss of lipogenesis and its relation to the parasitic lifestyle were tackled in this thesis: i) Has co-evolution between host and parasitoid led to the evolutionary loss of lipogenesis? ii) Which mechanisms underlie the loss of this essential metabolic trait in parasitoids? iii) How does dietary intake of nutrients affect life histories of organisms lacking lipogenesis? To answer the first question the link between parasitism and lack of lipogenesis was tested using phylogenetic analyses. 24 parasitoids were tested for their lipogenic ability and data on lipogenic ability of 70 other insect species obtained from the literature. The evolutionary loss of lipogenesis was found to have occurred in parallel in three different insect orders, all adopting the parasitoid lifestyle. This study further showed that lipogenesis had re-evolved in three parasitoid lineages that were characterized by their broad host range, i.e. generalists. Environmental compensation of lipid reserves by the host has led to the evolutionary loss of lipogenesis in parasitoids, yet this trait seems to re-evolve with relative ease, particularly in species that are expected to lack host manipulation to increase the host’s lipid resources.

Parasitoids that are specialized on one or only few hosts typically lack lipid synthesis, presumably because host manipulation substantially increases lipid resources of the host. Alternatively, in generalists adopting a large host range host manipulation is likely prohibited leading to a lower level of resources that can be taken over from the host. There are, however, exceptions to this general pattern, in which generalists have been found to lack lipogenesis. In one of these species, *Nasonia vitripennis*, host manipulation does occur, in which lipid levels are increased, hence this species is specialized at least to some extent on a number of hosts it prefers to use for oviposition. Another parasitoid adopting a large host range and lacking lipogenesis is *Pachycerepideus vindemmiae*. By determining lipid reserves of host and parasitoid this species was shown to lack host manipulation, hence the loss of lipogenesis is not inevitably related to host manipulation ability. At least in this species, mechanisms other than manipulation have led to the lack of lipogenesis, such as sufficiency in teneral reserves to maintain fitness or because the ability to manipulate the host has been lost.
One assumption regarding the lack of lipogenesis is that it involves only a lack of fatty acid synthesis, hence elongation and desaturation of fatty acids remains possible for metabolising other lipid types. By determining efficiency of host exploitation in a gall wasp community, it was found that at least one parasitoid species was able to adjust certain fatty acids ratios. This work further showed that all of the species tested within this community lacked lipid synthesis and that the majority of species showed an extraordinary efficiency in carrying over resources from their host. This is the first study to show that a parasitoid can adjust its fatty acid composition despite lack of lipogenesis.

While more cases of trait loss are currently being discovered, the mechanisms underlying the loss of traits have largely remained elusive. Studying transcriptional changes in response to food in the parasitoid *Nasonia vitripennis*, it was found that the transcriptional profile of this parasitoid severely deviates from that of *Drosophila melanogaster*, a species that actively synthesizes lipids. A lack of transcription of the key gene in fatty acid synthesis, fatty acid synthase (*fas*), explains the lack of lipogenesis in parasitoids. Lack of lipogenesis is not due to mutation in the coding region of this gene, because inspection of the amino acid sequence revealed no irregularities when compared to sequences of species that synthesize lipids. Lack of lipogenesis likely results from alterations in gene regulation, either through its response to hormones, non-functionality of transcription factors or deviations in other regulatory mechanisms involved in activating genes within the fatty acid synthesis pathway.

The acquisition of sufficient nutrient reserves is particularly important in organisms that are metabolically compromised, such as parasitoids. It can therefore be expected that nutrient sources with high caloric values are favoured over lower quality resources. In most organisms, however, a negative correlation has been found between a high-calorie diet and lifespan. In that sense, parasitoids lacking lipid synthesis are expected to differ from this general pattern, since consuming food sources with a higher caloric value should reduce the demand for limited lipid reserves and therefore correlate positively with life history traits. Using a two-pronged approach to discover the relationship between calorie intake, longevity and fecundity, two parasitoid species were subjected either to various dietary sugar dilutions or intermittent sugar-feeding. Dietary dilutions led to a typical caloric restriction effect, in which longevity decreased at higher sugar concentrations, while fecundity remained stable. When sugar sources were accessed intermittently, no effect of calorie restriction was found. These findings suggest
that, contrary to expectations, parasitoids do not benefit from feeding on a high calorie diet.

Parasitoids are frequently employed as natural enemies to fight pests in agro-ecosystems, but sugar sources are typically scarce within those environmental settings. Because sugar sources are scarce parasitoids can potentially benefit from feeding on a lipid-rich substrate as adults to increase their lipid reserves. Inclusion of a lipid-substrate in the diet of the parasitoid *Cotesia glomerata* led to an increase in lipid reserves or levels were maintained at a high level for a longer period of time. However, a lipid-rich diet significantly decreased longevity, either due to toxicity or because caloric values detrimentally affected longevity. Even though this experiment was only partly successful, it could be worthwhile to further explore optimal ratios for lipid provisioning to increase lipid resources benefiting allocation into longevity and reproduction.

A wide variety of traits have been lost during the course of evolution. It remains unclear however why trait loss occurs and how trait loss contributes to evolutionary trait dynamics within an ecological framework. What is clear is that phenotypic decay substantially adds to the component of genetic variation that remains hidden from selection and cryptic genetic variation plays a key role in both trait acquisition and re-evolution of lost traits. Therefore, trait loss plays an important role in facilitating the molecular resources that can be reacquired to extend the part of genetic variation expressed to the phenotype followed by selection and evolutionary change. Once we get more insight into the variety of mechanisms underlying trait loss we can entangle why and how trait loss contributes to evolutionary trait dynamics and reveal the importance of trait loss in evolutionary processes.