

A functional genomic investigation of an alternative life history strategy

The Alba polymorphism in *Colias croceus*

Alyssa Woronik

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Abstract

Life history traits affect the timing and pattern of maturation, reproduction, and survival during an organism's lifecycle and are the major components influencing Darwinian fitness. Co-evolved patterns of these traits are known as life history strategies (LHS) and variation occurs between individuals, populations, and species. The polymorphisms underlying LHS are important targets of natural selection, yet the underlying genes and physiological mechanisms remain largely unknown. Mapping the genetic basis of a LHS and subsequently unraveling the associated physiological mechanisms is a challenging task, as complex phenotypes are often polygenic. However, in several systems discrete LHS are maintained within the population and are inherited as a single locus with pleiotropic effects. These systems provide a promising starting point for investigation into LHS mechanisms and this thesis focuses on one such strategy - the Alba polymorphism in *Colias* butterflies. Alba is inherited as a single autosomal locus, expressed only in females, and simultaneously affects development rate, reproductive potential, and wing color. Alba females are white, while the alternative morph is yellow/orange. About 28 of 90 species exhibit polymorphic females, though whether the Alba mechanism and associated tradeoffs are conserved across the genus remains to be determined. In this thesis I primarily focus on the species *Colias croceus* and integrate results from lipidomics, transcriptomics, microscopy, and genomics to gain insights to the proximate mechanisms underlying Alba and Alba's evolution within the genus. Lipidomics confirm that, consistent with findings in New World species, *C. croceus* Alba females have larger abdominal lipid stores than orange, an advantage which is temperature dependent and arises primarily due to mobilized lipids. Gene expression data suggests differences in resource allocation, with Alba females investing in reproduction rather than wing color, consistent with previous findings in other *Colias* species. Additionally, I identify a morphological basis for Alba's white wing color. Alba females from *C. croceus*, an Old World species, and *Colias eurytheme*, a New World species both exhibit a significant reduction in pigment granules, the structures within the wing scale that contain pigment. This is a trait that seems to be unique to *Colias* as other white Pierid butterflies have an abundance of pigment granules, similar to orange females. I also map the genetic basis of Alba to a single genomic region containing an Alba specific, *Jockey-like* transposable element insertion. Interestingly this transposable element is located downstream of *BarH-1*, a gene known to affect pigment granule formation in *Drosophila*. Finally, I construct a phylogeny using a global distribution of 20 *Colias* species to facilitate investigations of Alba's evolution within the genus.

Keywords: *alternative life history strategy, butterfly wing color, Alba, Colias, color polymorphism, dimorphism, life history traits, physiology, lipidomics, genomics, pooled sequencing, phylogenomics, RNA-Seq, Pool-Seq.*

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To my family.

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Papers in this thesis

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

- I **Woronik, A.** & Wheat, C.W, (2016). Advances in finding Alba: the locus affecting life history and colour polymorphism in a *Colias* butterfly. *Journal of Evolutionary Biology* 30:26-39.
- II **Woronik, A.**, Neethiraj, R., Lehmann, P., Celorio-Mancera, M.P., Stefanescu, C., Hill, J., Käkälä, R., Brattstrom, O., Wheat, C.W. A transposable element insertion is associated with a female-limited, alternative life history strategy. *Manuscript*
- III **Woronik, A.**, Stefanescu, C., Käkälä, R., Wheat, C.W., Lehmann, P., Physiological differences between female limited, alternative life history strategies: the Alba phenotype in the butterfly *Colias croceus*. *Manuscript*.
- IV **Woronik, A.**, Kawahara, A., Warren, A., Chichvarkhin, A., Wheat, C.W., A phylogenetic analysis of butterflies in the genus *Colias*. *Manuscript*.

Paper I is reprinted with permission from the publisher

Candidate’s contribution

	I	II	III	IV
Conceived the study	Minor	Substantial	Substantial	Significant
Designed the study	Minor	Substantial	Substantial	Substantial
Collected the data	Substantial	Substantial	Substantial	Substantial
Analysed the data	Substantial	Substantial	Substantial	Substantial
Manuscript preparation	Substantial	Substantial	Substantial	Substantial

*** Contribution Explanation**

Minor: contributed in some way, but contribution was limited.

Significant: provided a significant contribution to the work.

Substantial: took the lead role and performed the majority of the work.

Introduction

A life history refers to the timing and pattern of maturation, reproduction, and survival during an organism's life cycle. The following are considered to be classic life history traits that directly impact the two major components of Darwinian fitness, survivorship and reproduction (Stearns, 1992):

- size at birth
- growth patterns
- age and size at maturity
- number, size, and sex ratio of offspring
- age and amount of investment in reproduction
- age and size at mortality
- lifespan

Patterns of co-evolved life history traits are known as life history strategies and variation in strategy occurs between individuals, populations, and species. Life history research aims to understand how selection pressures, either due to the environment or internal trade-offs and constraints, shape a life history strategy by optimizing life history traits to achieve maximum fitness. Recently there has been an increased interest in understanding the mechanisms underlying life history traits and strategies (Flatt & Heyland, 2011). Such knowledge would enable the investigation of fundamental questions regarding life history evolution. Examples of such questions include, but are not limited to:

- *What are the genes that underlie ecologically relevant life history traits within natural populations, and their functions?*
- *How do genetic and physiological mechanisms interact with the environment to effect expression of trade-offs?*
- *Are the genes and pathways that underlie the life history strategy conserved across taxa or lineage specific?*

However, uncovering these mechanisms is no easy task, as life history traits are often polygenic, which makes mapping the genetic basis and understanding the effects of those genetic regions on the phenotype very difficult.

Nonetheless, as developmental and molecular genetic techniques become more feasible there has been an increasing effort to take on this challenge. Many of the advances in this work focus on the mechanisms underlying the life history trait lifespan and are conducted in model species (yeast *Saccharomyces cerevisiae*, flies *Drosophila melanogaster*, and worms *Caenorhabditis elegans*) within the laboratory. For example, it is now known that much of the aging process is regulated by metabolic signalling pathways and transcription factors. Specifically, genes in the insulin and insulin-like signalling pathway have been shown to affect life span across several species indicating that certain key regulators of life history traits are evolutionarily conserved mechanisms (Kenyon, 2010).

While such advances using mutational, transgenic, and genomic approaches in model species have furthered our understanding of the mechanisms underlying life history traits, whether the genes identified using these approaches actually contribute to life history evolution in the wild remains unknown. Genes of importance in laboratory settings may be completely unimportant within natural populations, as selection acts upon variation in the wild rather than knockouts of candidate genes in specific pathways (Flatt & Heyland, 2011). Therefore the evolutionary conclusions that can be drawn from studies that identify the mechanistic basis of a life history trait in a model species within the laboratory are potentially biased and limited. This issue has historically been addressed in two ways. One can start with the gene, assessing the natural allelic variation that occurs at a candidate locus that was identified in the lab or one can start with the phenotype and characterize the genetic basis of a life history trait with known importance in the wild. For the latter approach, quantitative trait locus (QTL) mapping offers insights to the genetic architecture of the trait (i.e. many loci of small effect versus a single locus of large effect) and using finer scale mapping, potentially the gene or even a single nucleotide polymorphism (SNP) underlying the variation can be identified (Flatt & Heyland, 2011). With the advent of next generation sequencing, QTL mapping traits of interest has become increasingly accessible and provide great promise for increasing our understanding of the mechanisms underlying life history traits and strategies within wild populations and their evolution.

The challenges associated with uncovering the mechanisms underlying life history traits are only amplified when investigating the mechanisms underlying life history strategies and their evolution. However some traits are unique in that an entire suite of traits is inherited as a single locus, wherein individuals exhibit discrete, rather than continuous, variation in a life history strategy (Lank *et al.*, 1995; Gross, 1996; Andres & Cordero, 1999; Tsubaki, 2003; Küpper *et al.*, 2015; Lamichhaney *et al.*, 2015). Systems with this type of simple inheritance provide a promising place to begin investigating the

mechanisms underlying life history strategies. One such strategy occurs within butterfly species in the genus *Colias*.

The Alba polymorphism in *Colias* butterflies

In about 28 of the 90 *Colias* species, females exhibit two discrete life history strategies that are associated with a change in wing colour (Limeri & Morehouse, 2015). One morph, known as Alba, exhibits white wings, while the other exhibits yellow/orange wings similar to conspecific males (Figure 1). Across the six species in which it has been investigated, Alba is inherited as a single, dominant, autosomal locus (Remington, 1954). This has led some to posit that the Alba mechanism is conserved across the genus – though this has not been formally tested in any way. Much of the work investigating the trade-offs and selection dynamics acting on the two morphs has occurred in North American species. In *Colias eurytheme* the white wing colour of Alba females arises due to a large reduction in the amount of pteridine pigments in their wings. Alba exhibits a 20-fold reduction in sepiapterin (orange), 7-8 fold less xanthopterin (yellow), and a 3-fold reduction of erythropterin (red) compared to orange females while only exhibiting a slight increase in the colourless pteridine, leucopterin (Watt, 1973). Due to this decrease in pteridine synthesis Alba females reallocate resources, ~0.4 mg guanosine triphosphate (GTP) which equates to several percent of their nitrogen budget, to other areas of somatic and reproductive development (Watt, 1973).

Presumably as a result of this reallocation Alba females exhibit several advantages that lead to increased fecundity. However, the strength of this advantage varies in relation to several abiotic and biotic factors. Across all age classes wild caught *Colias scudderi* and *Colias alexandra* Alba females exhibit significantly larger fat bodies than orange females (Graham *et al.*, 1980). The fat body is a tissue unique to insects that plays a major role in multiple metabolic functions (Arrese & Soulages, 2010). In butterflies, fat body size is a strong predictor of fecundity (Boggs, 1981) and can also affect



Figure 1 Colour variation within *Colias croceus*. A) Alba female B) orange female C) male. Photo credit A. Woronik

adult survivorship (Graham *et al.*, 1980). In cold temperatures, Alba females of *Colias eurytheme* exhibit significantly faster pupal development (4% faster) and emerge with more mature eggs than orange females, while no such difference is observed in warmer temperatures (Graham *et al.*, 1980). This suggests that Alba has a selective advantage in cold conditions, a prediction supported by field observations. For example Hovanitz (1950) notes that Alba frequencies increase with elevation and latitude, suggesting Alba performs better in habitats with colder temperatures. Consistent with this, field experiments conducted by Nielsen and Watt (1998) find that in *C. scudderi*, a species that inhabits a cold environment and feeds on nitrogen poor host plant, Alba females have increased fecundity and occur at a higher frequency compared to *C. alexandra*, a species that lives in warmer conditions and feeds on a more nutrient rich host plant.

Alba's fitness also interacts with a male mating bias. In *Colias* butterflies wing colour is important for mate recognition. Studies of *C. scudderi* and *C. alexandra* show that males preferentially court orange females (Graham *et al.*, 1980). The strength of the bias corresponds to the co-occurrence with other white Pierid butterflies, with a strong bias against Alba found in populations where *Colias* frequently fly with other white species (Nielsen & Watt, 2000). While male discrimination against white butterflies increases their attention to conspecific females that are orange, this likely has negative fitness effects on Alba for two reasons. First, it may delay an Alba's first mating which could in turn affect her overall reproductive output. Second, males transfer nutrient rich spermatophores during matings (Boggs & Watt, 1981), and therefore Alba females may be unable to benefit from these additional nutrient reserves. Importantly, this male bias is also likely to be advantageous for Alba females, as they will spend less time and energy avoiding male harassment, allowing for those resources to be used for reproduction (Nielsen & Watt, 1998). In sum, the selection pressures acting on Alba arise from a combination of abiotic and biotic interactions, which likely contribute to the maintenance of the polymorphism within populations.

Interestingly, only about one third of *Colias* species exhibit polymorphic females, the remaining are monomorphic and exhibit either Alba or yellow/orange females (Limeri & Morehouse, 2015). The ancestral state of the genus is predicted to be polymorphic, since the sister genus *Zerene* also exhibits Alba females, therefore the monomorphic species likely arise due to a loss of polymorphism. Furthermore, the shift to monomorphism does not seem to consistently favour a single morph as they are lost at relatively equal frequencies (Limeri & Morehouse, 2015). If in fact Alba arose due to a single evolutionary event and the mechanism is conserved across the genus, then it would also be an excellent model to study how the environment interacts with the mechanisms underlying life history strategies to affect their

evolution. Alternatively, if Alba evolved repeatedly in the genus this system is ideal for investigating what environmental factors drive the repeated evolution of alternative life history strategies.

Study species

Most work in this thesis focuses on the butterfly *Colias croceus* (Geoffroy, Lepidoptera: Pieridae) (Figure 1), a wide ranging species found across Europe, Northern Africa, and the Middle East (D'Abrera, 1990). Non-Alba females have orange wings, similar to conspecific males. Crossing experiments confirmed that Alba in *C. croceus* is a dominant, single-autosomal locus, similar to the other species in which it was investigated (Remington, 1954). Alba's population frequency in *C. croceus* ranges between 5% and 30% across the Palearctic (Watt, 1973; Ford, 1975), and physiological studies document differences in resource allocation between the colour morphs (Descimon & Penner, 1989).

A brief overview of butterfly wing colour

The butterfly wing is charismatic structure that exhibits some of the most diverse colour patterns in the animal kingdom and has long fascinated both scientists and laymen alike. Due to this sustained interest, the evolution, development, and mechanisms underlying wing patterns and colour are relatively well understood. Wing colours can arise either via structures or pigments within the scale, a thin and flattened cuticular protrusion from specialized cells in the wing epithelium. Scales develop during the first few days of pupation and are tiled in

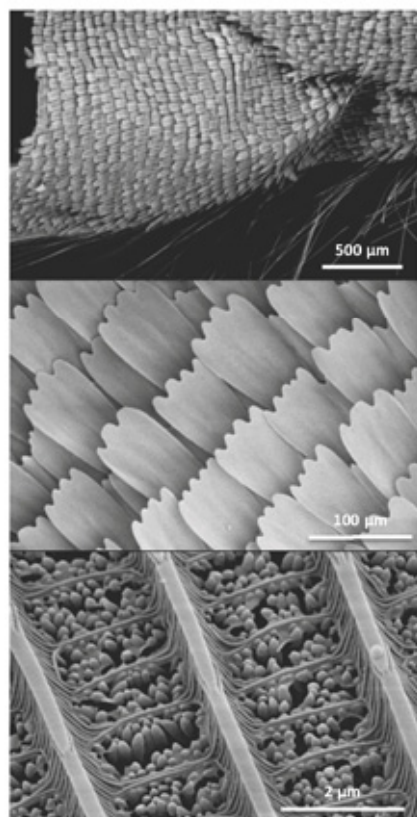


Figure 2 Visualization of a *Colias croceus* orange female wing using a scanning electron microscope at varying magnifications. Photo credit A. Woronik

a partially overlapping, shingle-like fashion across the wing (Figure 2). Scale morphology can differ dramatically among species. Each scale exhibits only a single colour and mosaics of these single coloured scales give rise to the large variety of colour we see on the wings (Nijhout, 1991).

Four major classes of chemical pigments give rise to the majority of wing colours: melanins, ommochromes, flavonoids, and pterins. Melanins are the most common, appear black or brown, and are distributed throughout the cuticle of the scale via diffusion. Ommochromes are red/brown pigments that are derived from tryptophan. Flavonoids cannot be synthesized by butterflies, and are instead obtained from the host plant. Colours that arise from flavonoid pigments include yellow, red, and blue. Finally pterins, derived from guanosine triphosphate (GTP), create varying shades of white, yellow, orange, or red colour patterns (Nijhout, 1991). Pterins colour the wings of *Colias* butterflies and are stored within the pigment granules of Pierid butterflies (Watt, 1964; Morehouse *et al.*, 2007).

Wing colour can also arise when light interacts with physical structures covering the scale. Structural colour and iridescences in butterflies arises via modification of three different structural components of the scale: the ridges, the microribs, and the lamina (Figure 3). White coloration is the most common structural colour and the most relevant for this thesis. White arises when the scale has many small and irregular surfaces that reflect light. The character of the white colour (i.e. chalky or pearly) depends on the regularity of the structures, which thereby affects the intensity of the scattered light. When the scale lacks pigment it will appear white due to light reflection off the ridges (Nijhout, 1991).

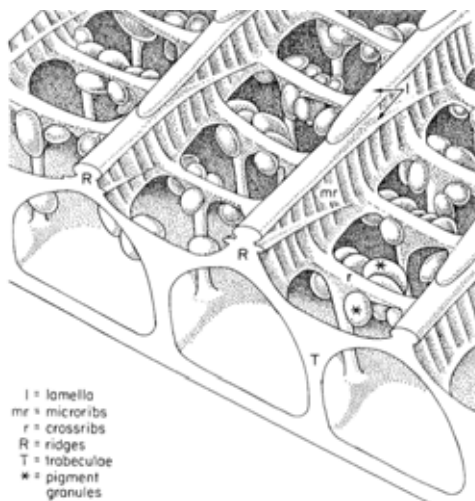


Figure 3 Diagram from Ghiradella, 1989 depicting the structure of a wing scale.

Aims of the thesis

The overall aim of the thesis is to fill key knowledge gaps in the Alba literature in order to gain a better understanding of Alba's mechanistic basis and evolution. The specific aims were to:

- Map the genetic basis of Alba within the Old World species *Colias croceus* (**Paper I and II**)
- Explore the proximate mechanisms underlying Alba and its associated trade-offs using electron microscopy, RNA-Seq, and lipidomics (**Paper II**)
- Delve deeper into the physiological trade-offs associated with Alba in *C. croceus*, by measuring differences in life history traits and examining potential gene by environment interactions (**Paper III**)
- Investigate whether traits associated with Alba are conserved between Old and New World species, suggesting a single evolutionary origin (**Papers II and III**)
- Construct a phylogeny for the genus *Colias* to facilitate future study of Alba's evolution (**Paper IV**)

Methods

Paper I used Pool-Seq data from a female informative cross in a bulk-segregant analysis to map the chromosome carrying Alba in *C. croceus*. Genes along the chromosome were then scanned for patterns of nucleotide diversity and SNP density consistent with being near the Alba locus. In **Paper II** a high quality *de novo* genome for *C. croceus* was constructed. Two additional bulk segregant analyses, using male informative crosses, and genome re-sequencing data from 15 Alba and 15 orange *C. croceus* females from wild populations were used to independently map the Alba locus to a single region on the previously identified chromosome.

Butterfly wing colour can arise either due to chemical pigments, scale structure, or a combination of both. Though the difference in pigment content between morphs had been investigated, potential differences in scale structure had not. **Paper II** investigated the differences in scale morphology between morphs by conducting electron microscopy on the wings of Old and New World *Colias* species, *C. croceus* and *C. eurytheme* respectively, and an extended sample of white Pierid butterflies.

Paper II also investigated the proximate mechanisms underlying aspects of the morph specific trade-off using RNA-Seq data from pupal wings and abdomens (4 individuals of each morph). A *de novo* transcriptome was constructed and annotated using gene ontology terms. The sixteen RNA-Seq libraries were mapped to the transcriptome and a differential expression and gene set enrichment analysis were conducted. Finally **Paper II** used High Performance Thin Layer Chromatography (HPTLC) on the abdomens of recently eclosed adults that had been reared in two temperature treatments to investigate whether aspects of the Alba trade-off identified by previous work in New World species were conserved in Old World species (i.e. that Alba females had larger abdominal lipid stores than orange and that the Alba advantage was stronger in cold conditions).

Paper III explores the physiological differences between the alternative strategies and the effect temperature has on their expression more deeply. **Paper III** used a split brood design, rearing full siblings at two temperature

treatments: hot (27°C) and cold (22°C during larval development and 15°C during pupal) and measured life history traits that have not before been compared between morphs (larval growth rate, adult wet weight, and abdomen to thorax ratio). Also for traits where differences between morphs had been previously reported (i.e. Alba's larger fat body stores and faster pupal development) **Paper III** delves deeper into trait characterization to better understand what potentially drives these differences. For example, HPTLC was used to quantify neutral lipid stores in the abdomen and dynamic injection respirometry was conducted throughout pupation to measure metabolic rate and the developmental budget (i.e. how much energy a developing pupa uses).

In **Paper IV** phylogenies were constructed from a global sampling of 20 *Colias* species. Both Bayesian methods using BEAST (Bouckaert *et al.*, 2014) and maximum likelihood methods using RAxML (Stamatakis, 2014) and maximum pseudo-likelihood using MP-EST (Liu *et al.*, 2010) were used on 72 hand-curated single copy orthologs to infer species relationships and divergence time estimates.

Major findings and conclusions

Paper I identified the chromosome carrying Alba in *C. croceus* as orthologous to chromosome 15 in *Bombyx mori*. Subsequent scans for selection along the chromosome revealed that divergence patterns consistent with being near to the Alba locus were localized to an ~ 5.7 Mbp region at the 5' end of the chromosome.

Paper II independently fine maps the Alba locus to the same region identified in **Paper I** using two male informative crosses and genome re-sequencing data from wild Alba and orange females (Figure 4a). Sixty-nine SNPs that were significantly associated with Alba occurred on an approximately 430 kb contig within and flanking a Jockey-like transposable element (Figure 4b). Based on read depth and alternative haplotype construction the transposable element is Alba specific (Figure 4d). Regions of sequence conservation between the Alba and orange haplotypes flank and are interspersed between two large and two small insertions (Figure 4e). The Alba insertions are located ~30 kb upstream of a *DEAD-box helicase*, and ~6kb downstream of a homeobox gene, *BarH-1* (Figure 4b).

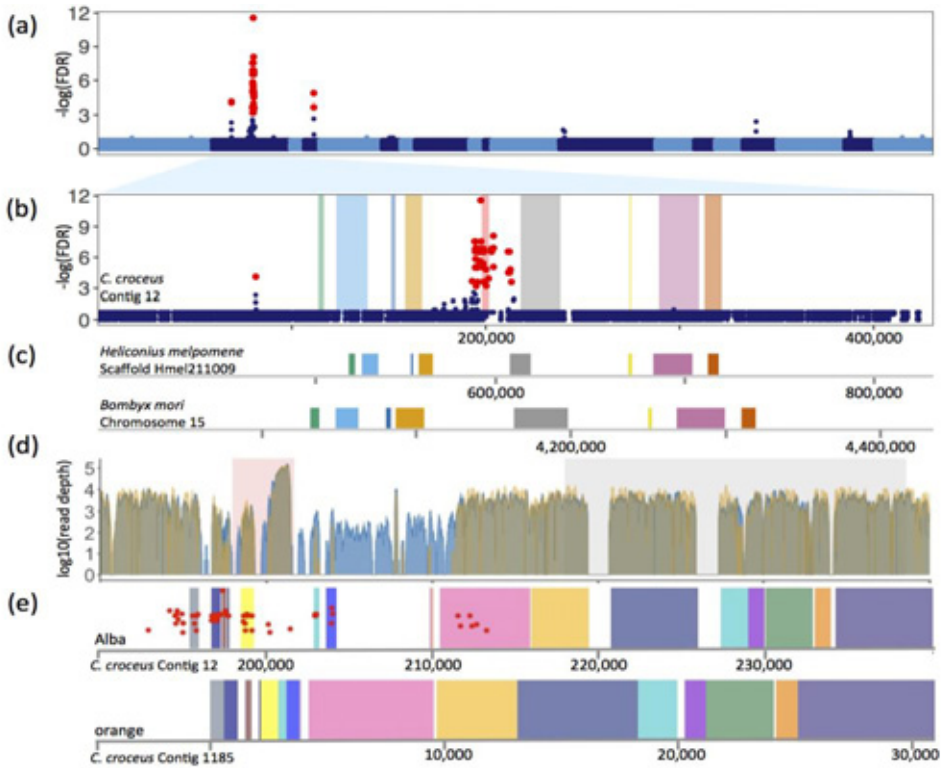


Figure 4 The Alba locus in *C. croceus* A) SNPs that significantly associate with Alba based on the GWAS across the locus identified in the bulk segregant analyses. The contigs that make up this region are shown in alternating dark and light blue. Red points are significant SNPs. B) The location of Alba associated SNPs on the ~430 kb outlier contig identified in the GWAS. Colour bars indicate the location of genes along the contig. The red block contains the *Jockey-like* TE exons, gold block is the *DEAD-box helicase* and the grey block is *BarH-1*. C) Comparing gene order within the *C. croceus* Alba locus to gene order in *Heliconius melpomene* and *Bombyx mori*. The conserved gene order among these species indicates a well-assembled region, as synteny is highly conserved within Lepidoptera. D) Read depth differences within the Alba locus for an Alba female (blue plot) and an orange female (orange plot). Red and grey blocks contain the *Jockey-like* TE and *BarH-1* respectively. Gaps in read depth within *BarH-1* are repeats, however they are not morph specific. E) Aligning locus haplotypes. Colour blocks indicate regions of conserved sequence. Top panel is the Alba haplotype while the bottom panel is orange. Red dots in the Alba haplotype indicate the Alba associated SNPs from the GWAS.

Paper II also investigates morphological differences between morph wing scales. Alba females from *C. croceus*, an Old World species, and *C. eurytheme*, a New World species both exhibit a significant reduction in pigment granules, the structures within the wing scale that contain pteridine pigments in Pierid butterflies (Figure 5). This is a trait that seems to be unique to *Colias* as three other species of white Pierid butterflies have an abundance of pigment granules, similar to orange females (Paper II Supplementary materials). This is an intriguing find as *BarH-1* knockout in *Drosophila* causes a colour change in the eye from red to white by affecting pigment granule formation (Higashijima *et al.*, 1992). In *Drosophila* the final stage of pigment synthesis occurs within the pigment granule (Mackenzie *et al.*, 2000). Therefore *BarH-1* may also have an important role in pigment granule morphogenesis, and thereby pigment synthesis and wing colour in *Colias*. To test that a reduction in pigment granules gives rise to a white wing phenotype in *Colias*, we chemically removed the pigment granules from the wing of an orange *C. croceus* female, which resulted in the formerly orange regions turning white. Further work using Crisper/Cas9 or RNAi methods to generate targeted knockouts would determine whether *BarH-1* gives rise to Alba via a lack of pigment granules.

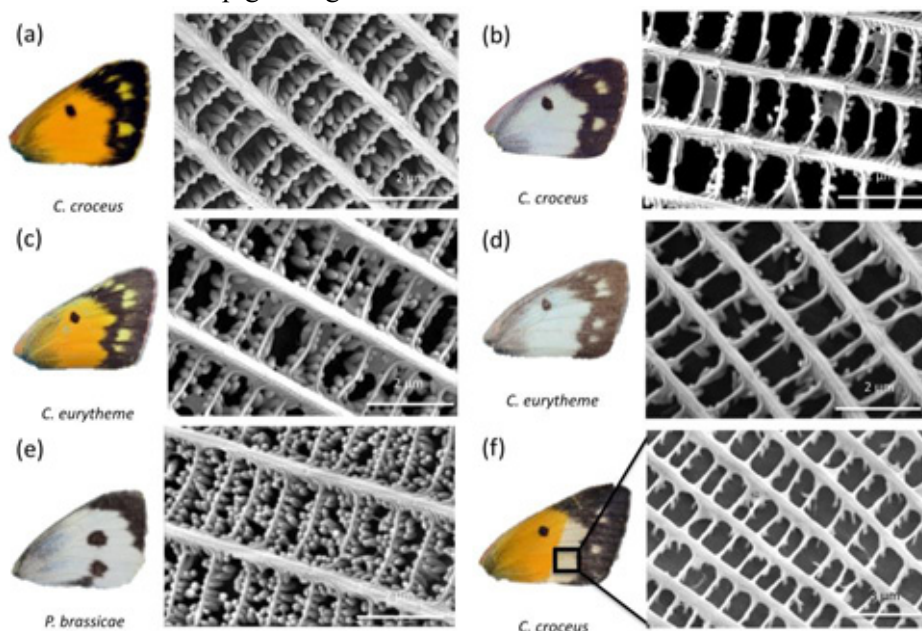


Figure 5 Differences in wing scale morphology between *Colias* colour morphs and *Pieris brassicae*. A) *C. croceus* orange female B) *C. croceus* Alba female C) *C. eurytheme* orange female D) *C. eurytheme* Alba female E) *P. brassicae* female F) *C. croceus* orange female where pigment granules have been chemically removed from half the wing. Microscopy image is from the white portion of the wing and illustrates the lack of pigment granules.

Furthermore **Paper II** explores the proximate mechanisms affecting the Alba trade-off using RNA-Seq data from pupal wing and abdomen tissue during the time of pigment biosynthesis. A gene set enrichment analysis revealed significant enrichment for 85 functional categories in abdominal tissue, including downregulation of ‘canonical Wnt signalling’, ‘regulation of Notch signaling pathway’ and ‘positive regulation of GTPase activity’ in Alba. Both Notch and Wnt pathways affect butterfly wing patterns in other species and may also play an important role in the Alba phenotype (Reed & Serfas, 2004; Martin & Reed, 2014). In wing tissue 35 categories exhibited significant enrichment including ‘regulation of transcription’ and ‘positive regulation of GTPase activity’ which were downregulated in Alba and ‘protein catabolic process’ which was upregulated. Enrichment for positive regulation of GTPase activity in both tissues is consistent with previous reports of GTP reallocation in Alba females (Watt, 1973).

Additionally differential expression analyses revealed *RIM*, a Rab GTPase effector (Pavlos & Jahn, 2011) is significantly upregulated in both tissues (log Fold Change (FC) of 3.4 in the abdomen and of 5.1 in the wings). RIM acts as a molecular switch by converting guanosine diphosphate to GTP, thereby activating its associated Rab GTPase, which is in turn involved in synaptic vesicle exocytosis and secretory pathways (Stenmark, 2009). RIM may play a role in the GTP reallocation observed in Alba females. Finally, consistent with findings that *C. eurytheme* Alba females emerge from the pupa with significantly more mature eggs (Graham *et al.*, 1980), *vitellogenin 1* was significantly upregulated within Alba abdomen tissue (logFC of 4.8). Vitellogenin is an egg yolk precursor protein that is synthesized in the fat bodies of insects (Hagedorn & Kunkel, 1979). These findings indicate that aspects of the Alba trade-off observed in New World species also occur within *C. croceus* lending further support to the hypothesis that the Alba mechanism is conserved across the genus.

Finally **Paper II** investigates physiological mechanisms underlying Alba using HPTLC on female abdomens to quantify the neutral lipid stores. Alba females had larger abdominal lipid stores than orange, however this advantage was temperature dependent and the difference was significant only within the cold treatment. This result is consistent with previous findings of Alba exhibiting larger fat bodies and higher fecundity than orange in colder conditions (Graham *et al.*, 1980; Nielsen & Watt, 1998).

Paper III delves deeper into morph specific physiological differences by quantifying life history traits. A lipidomics analysis using HPTLC revealed that in warm temperatures, though the overall level of lipid stores does not significantly differ between morphs, orange females do have significantly

more cholesterol and cholesterol esters (CE) than Alba females (Figure 6). Generally, the alcoholic (unesterified) cholesterol is located in cellular membranes and not in the storage lipid droplets, this therefore suggests that the orange morphs have higher membrane cholesterol content, which could translate to increased membrane integrity at elevated temperatures (Crockett, 1998). While in the cold treatment Alba females exhibited significantly larger CE, and diacylglycerols (DAG), and a trend for larger triacylglycerols (TAG) stores in storage droplets (Figure 6). These lipids can be mobilized for energy indicating of a capacity of growth and reproduction.

In the cold treatment, Alba females developed significantly slower than orange during the larval stage and had larger energy budgets during pupation (i.e. were more energetically expensive) (Figure 7). Contrary to previous findings, no significant difference in pupal development time was observed between morphs.

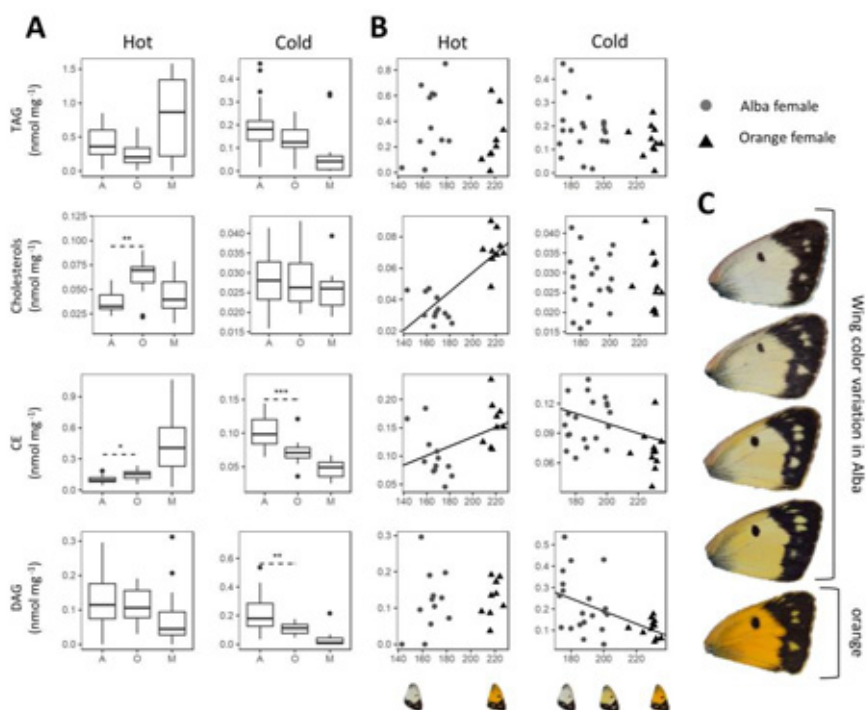


Figure 6 Analyses of major lipid classes (TAG, cholesterol, CE, and DAG) from abdomens of Alba females (A), orange females (O), and males (M). Though males are shown in the plots they were not included in statistical analyses. A) Box plots illustrating lipid differences between female morphs and males B) Regression of the mean red (wing colour) and the abdominal stores of neutral lipids. C) Wing colour variation in Alba individuals reared in the cold treatment compared to an orange individual reared in the cold treatment.

Finally, in **Paper III** an intriguing phenotype was observed within the cold treatment – yellow females (Figure 6 C). Upon further investigation yellow females appear to drive the significant difference between orange and Alba larval growth rate and developmental budget. When grouped into three morphs – Alba, yellow, and orange – a post hoc Tukey test revealed that yellow morphs developed significantly slower than orange in the larval stage and showed a trend for being slower than Alba, while Alba and orange did not significantly differ (Figure 7 C). Also only yellow and orange morphs significantly differed in their pupal developmental budget. An increased cost of development for the yellow phenotype may manifest as disruptive selection in the field, thereby favouring the maintenance of the two discrete morphs. Field observations support this hypothesis, as yellow morphs are extremely rare in wild *C. croceus* populations (personal observations of *C.*

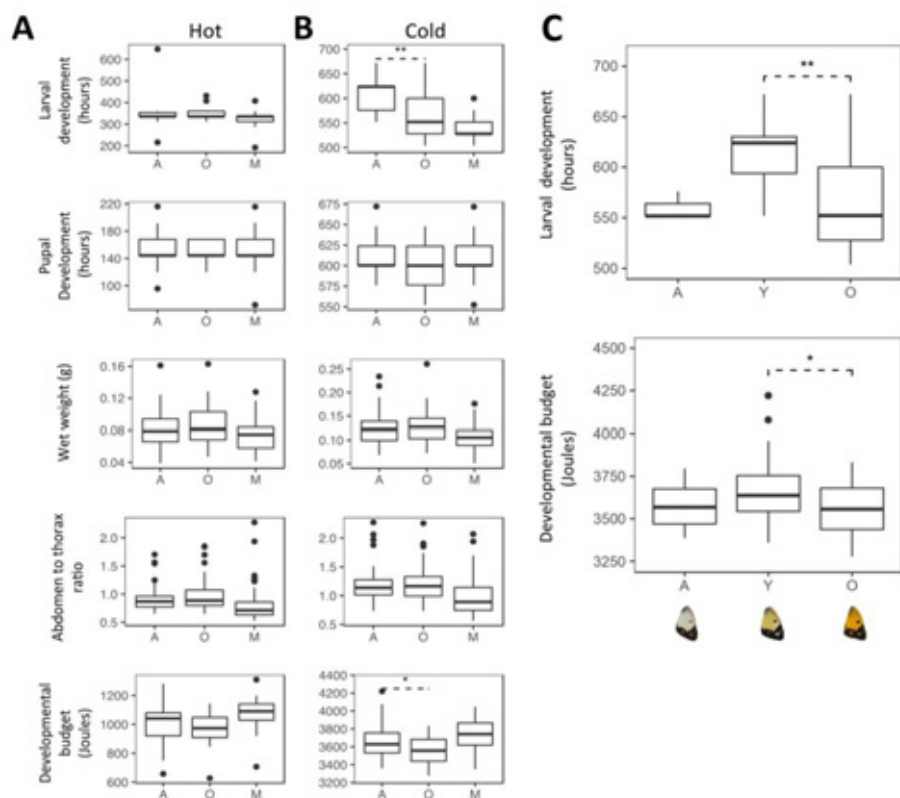


Figure 7 Major life history traits in *Colias croceus*. A) hot treatment B) cold treatment. Left to right A is Alba females, O is orange females, and M is males. C) A comparison of the major life history traits in *Colias croceus* where Alba and orange significantly differed in the cold treatment, larval development and pupal developmental budget. Females are classified into three morphs, Y is yellow

Stefanescu). Finally, cold temperatures have also been shown to induce a yellow phenotype in *C. eurytheme*, further suggesting a conserved Alba mechanism across the genus (Hoffmann, 1974) .

In **Paper IV** maximum likelihood/pseudo-likelihood and Bayesian methods were used to construct phylogenies for a global distribution of 20 *Colias* species. This is the most robust phylogenetic analysis of the genus to date, both in terms of number of genes analysed and the distribution of species. Though there was low branch support in the maximum likelihood analyses (Figure 8 B&C), likely due to a lack of phylogenetic signal in the dataset (Figure 9), all branches had a posterior probability of 1 in the Bayesian phylogeny. However these results should be taken with caution as Bayesian estimates of posterior probabilities can be too liberal (Suzuki *et al.*, 2002). Yet, some patterns remain consistent across all analyses, for example species grouped broadly by geographic range. There was a near complete agreement in a monophyletic grouping for South America (S.A.), Southern Eurasia/North Africa (S.E.N.A.), and North American (N.A.) taxa (Figure 8). Though some species relationships within the regional clades varied across analyses, five sister species relationships remained consistent.

Interestingly all three analyses indicated the New World species are polyphyletic (Figure 8). S.A. species are either sister to the rest of the genus (MP-EST and BEAST phylogenies) or to the S.E.N.A. clade (RAxML), while N.A. species appear to be founded by a common ancestor shared with *C. palaeno*, a circumpolar species. These results suggest that either the New World was colonized multiple times over the past 10 MY (Figure 8A), which is much older and previously envisioned (Wheat and Watt, 2008) or that *Colias* originated in the New World and shared a common ancestor with *Zerene*, the sister genus.

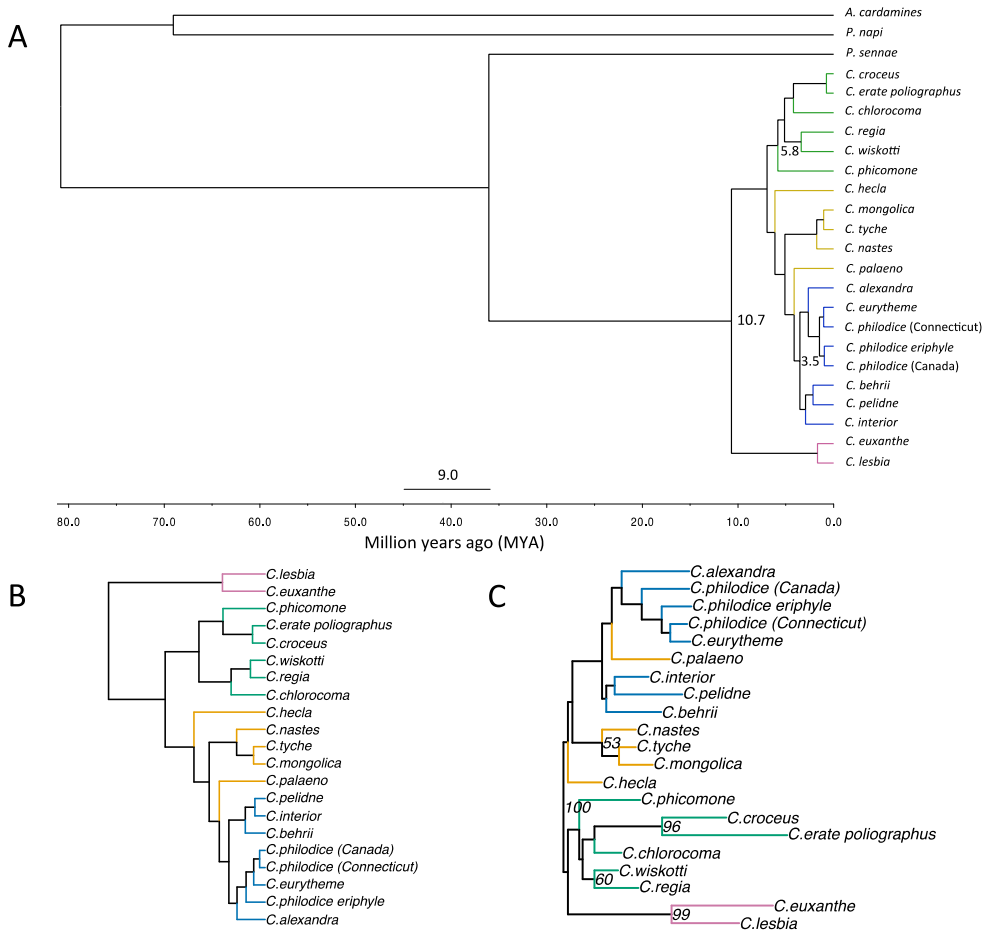


Figure 8 Phylogenies for *Colias*. Blue tips are North American species, yellow tips are Northern Eurasian, green are Southern Eurasian / North Africa, and pink are South America. A) Phylogeny estimated by BEAST using a concatenated matrix of 72 genes partitioned by codon site. Posterior probability for all nodes is 1. Node labels are divergence times in million years. B) Cladogram of maximum pseudo-likelihood estimated species tree by MP-EST. Outgroup species removed for better visualization of relationships within *Colias*. C) Phylogeny estimated by RAXML using a concatenated matrix of 72 genes partitioned by codon site. Outgroup species removed for better visualization of relationships within *Colias*. Node labels indicate bootstrap support, only bootstrap values greater than 50 are shown.

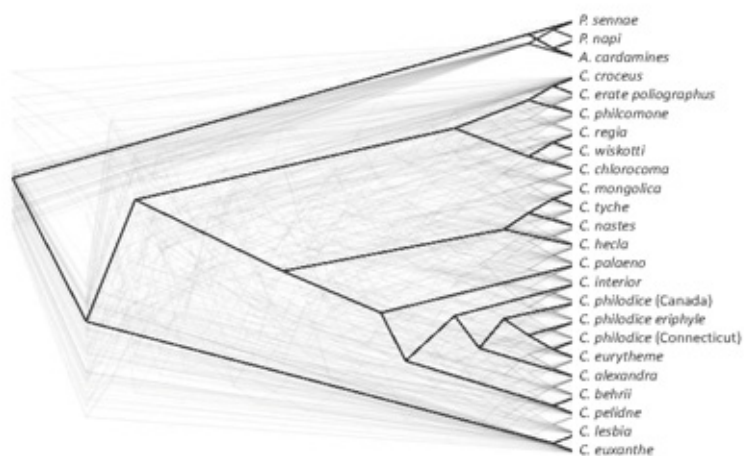


Figure 9 Cloudagram visualizing the gene trees underlying the species tree estimated by MP-EST illustrating the lack of a phylogenetic signal within *Colias*.

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Svensk sammanfattning / Swedish summary

Livshistorieegenskaper är faktorer som påverkar mönster som reproduktion och överlevnad hos organismer. De är de huvudsakliga komponenterna som ligger till grund för Darwinistisk fitness. Co-evolverade mönster hos dessa egenskaper kallas för livshistoriestrategier (LHS). LHS kan variera mellan individer, populationer och arter. Variationen som ligger till grund för LHS är viktiga för naturligt urval, ändå är de genetiska och fysiologiska mekanismer som påverkar dem ofta okända. Kartläggning av den genetiska basen för LHS och vidare studier av associerade fysiologiska egenskaper är utmanande eftersom komplexa fenotyper ofta påverkas av många gener. I många system upprätthålls ändå LHS inom populationer och ärvs som ett enda locus med pleiotrofiska effekter. Sådana system utgör lovande utgångspunkter för studier av mekanismerna bakom LHS. Föreliggande avhandling fokuserar på en sådan strategi - Albapolymorfismen hos fjärilar av genuset *Colias*. Alba ärvs som ett autosomalt locus som endast uttrycks hos honor och har pleiotrofiska effekter på utvecklingshastighet, reproduktiv potential och vingfärg. Albahonor är vita, medan den alternativa morfen är gul eller orange. Ungefär 28 av 90 arter inom genuset uppvisar Albapolymorfiska honor, men det är ännu inte utrett om Albamekanismen och dess associerade tradeoffs är homologa eller ortologa inom genuset. I denna avhandling fokuserar jag primärt på arten *Colias croceus*. Jag integrerar resultat från lipidomik, transkriptomik, mikroskopi och genomik för att nå insikter om de proximala mekanismer som ligger till grund för Alba och evolutionen av Alba inom genuset. Lipidomikstudierna bekräftar att Albahonor hos arten *C. croceus* har större abdominala fettreserver, i linje med upptäckter i nya världen. Stora fettreserver är en fördel som är temperaturberoende och som framför allt uppkommer genom mobiliserade lipider. Data över genuttryck tyder på skillnader i resursallokering, där Albahonor investerar i reproduktion snarare än vingfärg, vilket är konsistent med upptäckter hos andra Coliasarter. Vidare identifierar jag en morfologisk grund för Albas vingfärg. Albahonor av *C. croceus* (en art från gamla världen) och *C. eurytheme* (en art från nya världen) uppvisar båda en signifikant minskning av pigmentgranuler, strukturen i vingarna som innehåller pteridinskt pigment. Detta är en egenskap som tycks vara unik för *Colias* då andra vita pierida fjärilar har mycket pigmentgranuler, i likhet med orangea honor. Jag kartlägger också den genetiska bakgrunden till Alba till en enda genomisk region som innehåller ett Albaspecifikt jockey-liknande

trasbosabelt element. Detta utgörs av en insertion som ligger nedströms från BarH-1, en gen med känd effekt på bildandet av pigmentgranuler i *Drosophila*. Slutligen konstruerar jag en fylogeni över den globala distributionen av 20 *Colias*arter för att kartlägga evolutionen av Alba inom genuset.

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