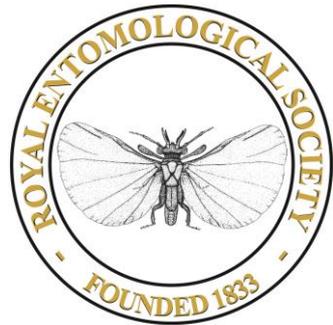
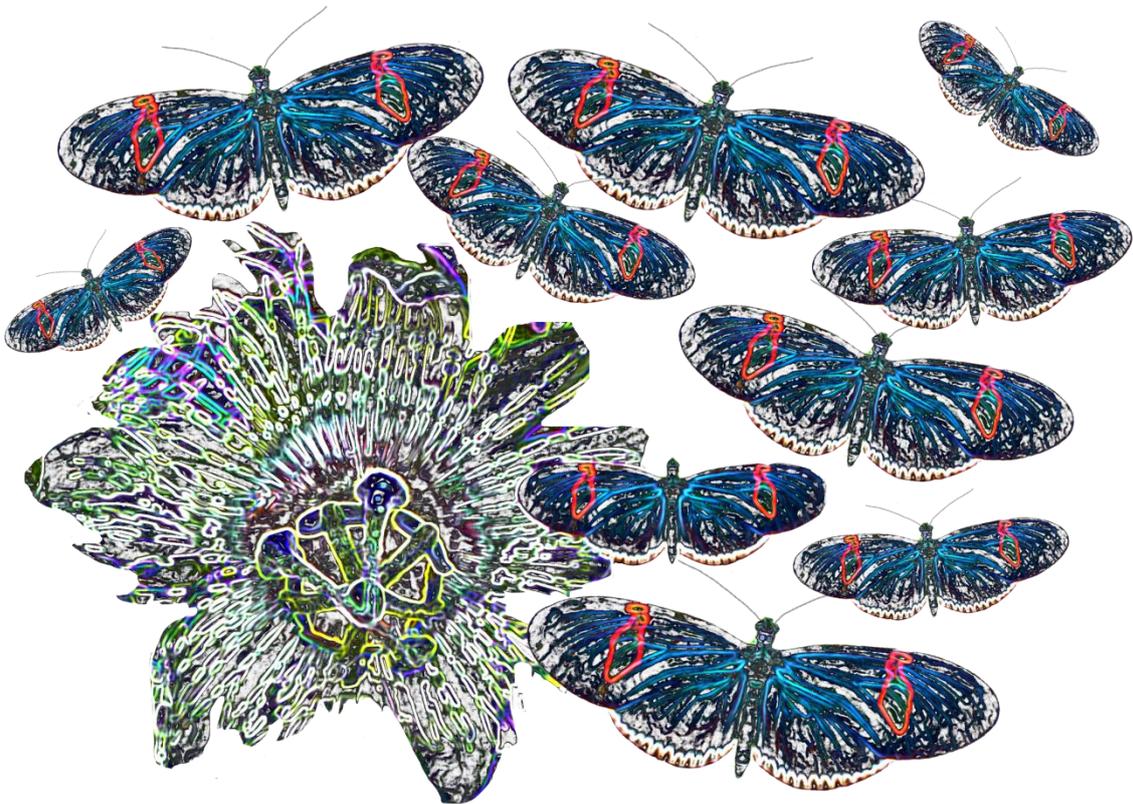




Heliconius

SHEFFIELD 2016



Species boundaries among *Heliconius* butterflies reflect the genetic architecture of speciation

Simon Martin

University of Cambridge

We still lack a detailed understanding of the genetic mechanisms that allow species to coexist and hybridize without collapsing. Theory predicts that genetic incompatibilities that reduce fitness of hybrids and recombinants may form barriers to gene flow, particularly in genomic regions of reduced recombination. This has proved difficult to test empirically. Conventional methods for studying the landscape of gene flow across the genome are prone to biases, making them difficult to interpret. We addressed this issue by using novel, and less biased, approaches to study relatedness across the genome between hybridizing species of *Heliconius* butterflies. We compared two pairs of species using multiple resequenced genomes from multiple replicated regions of sympatry. We found that relatedness between species fluctuates on a large scale across the genome, and that patterns of introgression differ between the two species pairs. In one pair, gene flow is correlated with recombination rate, reduced at chromosome centres and in gene-rich regions. In the other pair, rates of gene flow are more even across the genome. Using simulations, we show that these patterns are consistent with biological differences between the two species pairs. One pair has distinct wing patterns that are under strong ecological selection and may provide a genome-wide barrier to gene flow. The other pair lacks this dramatic ecological difference, so the species boundary depends more on the distribution of genetic incompatibilities and recombination. Our findings therefore supplement theoretical work, showing how the shape of the species boundary reflects the genetic architecture of species differences.

The comparative landscape of duplications in *Heliconius melpomene* and *Heliconius cydno*

Ana Pinharanda, Simon H. Martin, John W. Davey & Chris D. Jiggins

University of Cambridge

Gene duplications can facilitate adaptation and may lead to inter-population divergence, causing reproductive isolation. We used whole-genome re-sequencing data from 34 butterflies to detect duplications in two *Heliconius* species, *H. cydno* and *H. melpomene*. Taking advantage of three distinctive signals of duplication in short-read sequencing data, we identified duplicated loci in *H. cydno* and *H. melpomene*. Duplications overlap genes significantly less than random in *H. melpomene*, consistent with the action of background selection against duplicates in functional regions of the genome. We identified duplications that may be under selection, including several gustatory receptors and an odorant binding protein. These candidates merit further investigation for a potential role in host plant and mate recognition differences between the species.

Fine-scale recombination maps show no major inversions between sympatric *H. melpomene* and *H. cydno* populations

John Davey

University of Cambridge

One of the proposed explanations for the maintenance of species differences in sympatry is linkage of divergent alleles via chromosome inversions. Several examples of this mechanism have been demonstrated, particularly in *Drosophila*. However, recent models indicate that inversions are only likely to enhance the maintenance of species barriers in certain limited circumstances. I will present fine-scale recombination rates within *H. melpomene*, within *H. cydno* and between *H. melpomene* and *H. cydno* from Panama to demonstrate that there are no major inversions between these species, and suggest that this is consistent with theoretical results. I will also cover other interesting features of the fine-scale recombination maps, the first for Lepidoptera; for example, some pairs of chromosomes that were fused in the *Heliconius* lineage since the split between *Heliconius* and *Eueides* have markedly different recombination rates, suggesting that regulation of recombination has been preserved since the origin of the *Heliconius* genus and is not simply driven by chromosome length.

Inversion polymorphism changes the rules of speciation in a mimetic butterfly

Mathieu Chouteau, Violaine Llaurens, Annabel Whibley, Florence Prunier, **Mathieu Joron**

CNRS France

Mate choice is a key component of the dynamics of adaptive alleles in a population. Emphasis has been given to assortative mating where mating occurs between individuals with similar phenotypes, facilitating local adaptation and speciation. Less is known about disassortative mating (or negative assortative mating) defined by preferential pairings between individuals differing in phenotype. We investigate wing pattern polymorphism displayed by toxic butterflies (*Heliconius*). Their patterns are signals of toxicity learned by local predators, and enhance survival through predator avoidance and mimicry with other local toxic prey. Warning signals are often used as mating cues, driving assortative mating and speciation. However, the Amazonian species *Heliconius numata* maintains a stable polymorphism with multiple colour morphs coexisting within populations, a situation which mimicry alone or assortative mating cannot explain. Morphs are controlled by a single locus (supergene) formed by multiple chromosomal inversions. Using experimental mating trials we found strong disassortative mating between morphs, and showed this was mediated through female choice. The wing pattern supergene showed clear excesses of heterozygotes in natural populations, contrasting with an otherwise freely panmictic genome. This may be expected if chromosomal inversions carry recessive deleterious mutations, as is known for other supergenes, causing heterozygous advantage. Between populations, genomes were atypically undifferentiated across the continental distribution. We modelled the consequences of disassortative mating on polymorphism in spatially structured populations. Disassortative mating produced negative frequency-dependence, which favours rare morphs, including those bringing poor survival benefits (absence of local co-mimics) so long as they are recessive, which fits our empirical observations. Effective gene flow between populations was enhanced through mating benefits to recessive wing-pattern alleles, acting against population differentiation, and enhancing effective population size. Through both local and global effects on the mixing of genomes, disassortative mating at the supergene maintains adaptive polymorphism, and acts against speciation.

Supergene evolution favoured by the introgression of an inversion in *Heliconius*

Paul Jay, Annabel Whibley, Angeles de Cara, Mathieu Joron

CNRS France

Heliconius numata shows a spectacular polymorphism with multiple coexisting forms which mimic distinct local butterfly species (Ithomiines) and their geographic variations. Wing-pattern polymorphism is controlled by a group of tightly-linked genetic elements, or supergene, maintained in linkage disequilibrium by polymorphic chromosomal rearrangements. Each arrangement is characterised by one or several inversions, associated with a given phenotypic form. Here we investigate the origin of inversion polymorphism and the role of introgression in the formation of the supergene. Breakpoint genotyping in related species shows that the main inversion is shared with a non-sister species, *H. pardalinus*, suggesting introgression may explain the emergence of a new allelic class in *H. numata*. Based on whole genome resequence data, *f* and *D* statistics (ABBA-BABA analyses) reveal an excess of shared derived mutations in the inversion between *H. numata* and *H. pardalinus*. Treemix analyses indicate a history of gene flow between the two taxa, and topological changes in the phylogeny across the genome show a pattern of haplotype sharing consistent with an ancient introgression of the inversion into *H. numata*. Multiple sequentially Markovian *coalescent* (MSMC) analyses corroborate this ancient origin, while the population branching statistic (PBS) and haplotype similarity confirm the common origin of the inversion. Finally, comparative demographic inferences and Approximate Bayesian Computation (ABC) simulations lead to the hypothesis that supergene formation and polymorphism could be associated with an increase in effective population size and gene flow in *H. numata*. We conclude that the introgression of an inversion kick-started the evolution of the supergene and enabled distinct adaptive morphologies to coexist. Contrary to well-known cases where mimicry shifts cause speciation, here inversion polymorphism and demographic events may favour the maintenance of intraspecific diversity and inhibit cladogenesis.

Characterization of the wing colour patterning supergene in *Heliconius numata*

Suzanne Saenko

CNRS UMR7205

Identification and experimental validation of genes underlying adaptive phenotypic variation is crucial for our understanding of evolution. *Heliconius numata* is highly polymorphic for wing colour patterns, with 7-10 forms coexisting in the Amazon populations, each mimicking a different species of *Melinaea* butterflies. This dramatic polymorphism is controlled by alleles of a single supergene *P*, containing at least 18 genes. Polymorphic inversions at the *P* locus suppress recombination, maintaining the favourable genetic combinations that produce mimetic colour patterns. However, the exact nature of the wing patterning genes within the supergene has not yet been investigated. Here, we use RNA-sequencing in combination with 'classical' approaches such as qPCR and *in situ* hybridizations to test the involvement of candidate genes from the *P* supergene in wing pattern formation in *H. numata*. Remarkably, this locus harbors the genes involved in colour patterning in other species of Lepidoptera and therefore represents a genomic hotspot of evolutionary change in multiple clades.

Mimicry in the Making: the genetic basis of convergent evolution in *Heliconius* wing patterns

Joe Hanly

University of Cambridge

The wing patterns of *H. melpomene* and *H. erato* provide one of the most striking examples of repeated convergent evolution in nature. A small number of genes, including *optix*, *wntA* and *cortex*, have acted as "hotspots", upon which selection has repeatedly acted to generate highly mimetic wing patterns. Much of the genotypic variation associated with these differences maps to non-coding sequence, and therefore probably affects regulatory elements of hotspot genes, driving changes in their spatial expression profiles. We have now been able to identify broad modules associated with the three mimetic red pattern elements *dennis*, *ray* and *band*, in both species, through a combination of genotype association studies and phylogenetic analyses. The *dennis* module appears to have formed through modification of either the same or neighbouring conserved sequence in both species whereas the *ray* and *band* elements appear to have formed within different, non-homologous regions.

Genomic dynamics of speciation in *Timema* stick insects.

Victor Soria-Carrasco

University of Sheffield

Speciation can be initiated by few genetic changes. However, dynamics along the full speciation continuum are poorly understood. We report a gap in the speciation continuum based on ecological and genomic data from >100 populations of 11 species of *Timema* stick insects. We identify factors that promote bridging this gap, including genome-wide changes, mate choice, and time. Although major-effect loci do contribute to speciation, their effects are restricted unless they align with other factors, and genome-wide differentiation evolves early in speciation. Evolutionary gaps emerge when gene flow causes this multi-faceted process to change its pace or to be restricted in space. In the spectrum of speciation models, our results show a dynamic and genomic concept is as plausible as the often-emphasized gradual and genic one.

Reproductive isolation and geography in *Heliconius elevatus* and *Heliconius pardalinus*

Neil Rosser

University of York

Heliconius elevatus and *Heliconius pardalinus* occur throughout much of northern South America, and are sympatric in the Amazon basin. Whole genome analysis shows Amazonian *H. elevatus* and *H. pardalinus* to be sister species that differ only at a handful of divergent genomic regions. An allopatric race of *Heliconius pardalinus* from northern Andes, *H. p. sergestus*, comprises the sister to the Amazonian *elevatus/pardalinus* clade. In Peru, we have been testing prezygotic and postzygotic isolation between these taxa. Amazonian *H. elevatus* and *H. pardalinus* exhibit strong assortative mating, but hybrids between them are fully fertile. In contrast, *H. pardalinus* from the Amazon and *H. pardalinus sergestus* seem to mate randomly, but female F1 hybrids are sterile. I will discuss the implications of these findings in light of the taxa's geographic distributions. Our work currently focuses on trying to map the genes involved in prezygotic reproductive isolation between Amazonian *H. elevatus* / *pardalinus*, and those involved in postzygotic reproductive isolation between *H. pardalinus sergestus* / Amazonian *pardalinus*.

Characterising Reproductive Barriers Between Three Closely Related *Heliconius* Butterfly Taxa.

Lucie Queste

University of York

Debates about the possibility of reinforcement have been an ongoing feature in the field of speciation. However, recent theoretical studies and examples in nature have confirmed a role for reinforcement in speciation. Much research now focuses on finding more evidence such as stronger isolation in sympatric populations. *Heliconius* butterflies offer extensive opportunities to answer such questions. Here, I present two experiments, carried out in Peru, that aim to determine whether male colour pattern preference and female host plant preference act as reproductive barriers in three *Heliconius* taxa with varying degrees of geographic overlap. Two sympatric taxa are considered, *H. elevatus* and *H. pardalinus butleri*, along with a third taxa, *H. p. sergestus* which is allopatric to *H. p. butleri* but parapatric to *H. elevatus*. Results suggest that these traits play a role in the reproductive isolation observed between the taxa, however, the pattern of stronger isolation in sympatry was not as strong for colour pattern preference. These results provide a first understanding of the ecological factors involved in the evolution of these taxa and highlight the need to investigate further barriers involved in the reproductive isolation observed.

Genetic analysis of wing pattern and pheromone composition in two sister species of *Heliconius* butterflies.

Bruna Cama

University of York

In *Heliconius*, sister species which diverged with gene flow show genomic hotspots where the amount of genetic differentiation is higher than in the rest of the genome, which instead remains consistent with a scenario of complete admixture. Loci controlling traits causing reproductive isolation are expected to be found in these “islands of divergence”. The aim of this project is to identify and map the hotspots of divergence linked to colour pattern and male pheromone production in two sympatric sister species, *Heliconius pardalinus butleri* and *Heliconius elevatus*, with the aid of in-depth phenotypic analysis of F2 hybrids. These two traits, both important in mate recognition, differ noticeably between the two species,. F2 *H. elevatus* x *H. pardalinus* hybrids were used to score colour pattern phenotypes and male androconia pheromone blend composition (via GC/MS). Several putative Mendelian loci were identified for the colour pattern, most of which are distributed between two linkage groups, one for the orange elements and one for the white/yellow ones. The pheromone components appear organized in subgroups of correlated compounds whose biosynthesis may potentially be controlled by the same sets of genes.

Identifying the nature of mimicry among prey with unequal defences

Thomas Aubier, Mathieu Joron, Tom Sherratt

CNRS France

Understanding the mutualistic or parasitic nature of interspecific mimicry is central to explaining the diversity of warning signals in nature. Whether prey with unequal defences engage in parasitic ("quasi-Batesian") mimicry is widely debated. Experimental evidence is ambiguous, whereas models of predator learning generally predict quasi-Batesian relationships. However, predators' attack decisions are not based on learning alone but also on the future value of information, generating an exploration-exploitation trade-off. We identify the optimal sampling strategy of predators capable of classifying prey into different profitability categories. We show that, while increasing overall prey consumption, moderately unprofitable mimics typically cause a decrease in the mortality of highly unprofitable models as a result of an increase in signal frequency. Additionally, the rare instances of quasi-Batesian relationships are only weakly parasitic. Our analysis suggests that if predators forage efficiently, quasi-Batesian mimicry should be rare, which may explain the scarcity of evidence for it in nature.

You are what you eat: cyanogenic glucosides in *Heliconius* and closely related genera

Érika Castro^{1,2}, Helene Engler, Márcio Zikan Cardoso³, Lawrence Gilbert⁴, Mika Zagrobelny^{1,2} and Søren Bak^{1,2}

¹Department of Plant and Environmental Sciences, University of Copenhagen, Denmark;

²Copenhagen Plant Science Center (CPSC), University of Copenhagen, Denmark;

³Departamento de Ecologia, Universidade Federal do Rio Grande do Norte, Brazil; ⁴Section of Integrative Biology, The University of Texas at Austin, USA.

Heliconius butterflies and *Passiflora* plants are both toxic due to the presence of the defence compounds called cyanogenic glucosides (CNGlcs). When under attack, they both release poisonous cyanide as a result of the degradation of the CNGlcs by β -glucosidases. It has been suggested that the ability to synthesize the aliphatic CNGlcs linamarin (LIN) and lotaustralin (LOT) is a common trait of all *Heliconius* species. However, species of the *sara-sapho* group seem to have lost this ability and become specialized for the uptake of epivolkenin (EPI), a cyclopentenyl CNGlc from their host plants. Although the genus *Heliconius* has been intensively studied for the past 150 years, the cyanogenic profile of many species and closed related species is still unknown. Also, the contribution of host plant use during the larval stage to this chemical profile is unclear. The aims of this work were to establish the CNGlc profiles of *Heliconius* imagines as well as other species from the Heliconiinae subfamily and to elucidate which CNGlcs can be taken up from plants by *Heliconius* larvae. By feeding *H. melpomene* larvae with *P. platyloba* leaves painted with different types CNGlcs we observed that they are able to take up cyclopentenyl and aliphatic (radiolabelled marked) CNGlcs from their diet and keep them intact in their haemolymph. However, they are not able to do the same with aromatic CNGlcs. We also observed that *H. melpomene* larvae have some adaptations for the uptake of intact CNGlcs from the host plant, i.e. perform leaf-snipping during feeding to keep most of the leaf pieces intact and have an alkaline gut pH that inhibits β -glucosidase activity. Imagines of *Heliconius* and other genera of the *Heliconiinae* subfamily were analysed by LC-MS and they all contained LIN and LOT, except for *H. sapho* and *H. hewitsoni*. Instead, high amounts of EPI was detected in these species, indicating that both may have lost the ability to synthesize CNGlcs and become more specialized for sequestration of CNGlcs from their host plants, as suggested by (Engler-Chaouat & Gilbert, 2007). Cyclopentenyl CNGlcs were also found in all *Heliconius* and some of the closely related species, including species from *Acraeini* and *Argynnini* tribes. Thus, sequestration of cyclopentenyl CNGlcs probably appeared in a common ancestor of the Heliconiinae subfamily. In *Heliconius* this trait seems to be so crucial that some species have lost the capacity to synthesize CNGlcs, relying entirely on obtaining their chemical defence from their host plants. Our results suggest that the cyanogenic profile of these butterflies vary according to the chemical profile of their host plant in the larval stage. Our research group is currently characterizing the biosynthetic pathway of LIN and LOT in *H. melpomene* which will improve our knowledge about the balance between sequestration and biosynthesis of CNGlcs, and the evolution of this pathway in the Lepidoptera order.

Gene duplication and gene expression changes play a role in the evolution of candidate pollen feeding genes in *Heliconius* butterflies

Gilbert Smith, Aide Macias-Muñoz, Adriana D. Briscoe

University of California, Irvine

Heliconius possess a unique ability among butterflies to feed on pollen, which can significantly extend their lifespan and is thought to have been important to the diversification of the genus. We used RNA sequencing to examine feeding-related gene expression in the mouthparts of four species of *Heliconius* and one non-pollen feeding species, *Eueides isabella*. We hypothesized that genes involved in morphology and protein metabolism might be upregulated in *Heliconius* because they have longer proboscides than *Eueides*, and because pollen contains more protein than nectar. Using *de novo* transcriptome assemblies, we tested these hypotheses by comparing gene expression in mouthparts against antennae and legs. We first looked for genes upregulated in mouthparts across all five species and discovered several hundred genes, many of which had functional annotations involving metabolism of proteins, lipids and carbohydrates. We then looked specifically within *Heliconius* where we found eleven common upregulated genes with roles in morphology, behavior, and metabolism. Closer examination of these candidate genes revealed gene duplication events and expression regulatory changes along the lineage leading to heliconiine butterflies, and within *Heliconius*. Further, our results suggest that the butterfly proboscis might be directly involved in digestive enzyme production.

Coevolution of *Heliconius* butterflies: evidence and outstanding questions

Jennifer F. Hoyal Cuthill

University of Cambridge and Tokyo Institute of Technology

Until the late twentieth century, the *Heliconius* butterflies were often discussed as an example of coevolution, in the strict sense of reciprocal evolutionary change. This changed in the nineteen-nineties when phylogenetic analyses of mitochondrial DNA led to a rejection of coevolution between the key co-mimics *H. erato* and *H. melpomene*. Subsequent theoretical work then focussed on other routes to mimicry, such as advergence (secondary evolution of mimetic patterns in one co-mimic, for example *H. melpomene*). However, more recent phylogenies have provided new evidence for coevolution, suggesting that *H. erato* and *H. melpomene* are of similar ages, show correlated mimicry diversifications and have compatible phylogeographic histories. While it is difficult to definitively prove strict coevolution, this places the possibility firmly back on the table. This presentation will discuss the evidence for coevolution, its interpretation and potential implications for mimicry theory.

Population genomics of a multi-species mimetic hybrid zone

Jake Morris

University of York

Across the Guianas, seven species of *Heliconius* form intra-specific hybrid zones, with hind wing rays present in the Eastern Guianas but not in the Western Guianas. In the distantly related species, *H. melpomene* and *H. erato*, the same homologous locus 'Rays', has been found to control this colour pattern shift. Here we study two additional species, *H. demeter* and *H. aoede*. Using population genomics approaches we identify 'islands of divergence' in both species across the hybrid zone. Our results implicate a role for 'Rays' in both species. Thus parallel genetic evolution at this locus seems to have repeatedly contributed to colour pattern evolution across *Heliconius* taxa. However, in both *H. demeter* and *H. aoede* there are loci showing much greater divergence than that at 'Rays'. This suggests both may have evolved new, alternative solutions to this specific colour patterning problem, which work in combination with more ubiquitous 'Rays' locus.

Comparing parallel clines of a polygenic iridescence trait in mimetic butterflies

Emma Curran*, Melanie Brien*, Carolina Pardo-Diaz, Camilo Salazar, Roger Butlin*, Nicola Nadeau*

*University of Sheffield

The *Heliconius* butterflies of South and Central America are well-known for their brightly coloured mimetic warning patterns. The rich diversity in wing patterning in these species is the result of a handful of major effect loci. These loci control various colour pattern elements produced by pigments. In the west of Colombia and Ecuador, the co-mimics *Heliconius erato* and *Heliconius melpomene* display these pattern elements against a backdrop of iridescent blue, a colour produced by the scattering of light from intricately layered nano-scale structures on the surface of wing scales. In contrast to pigment colours, evidence from crosses suggests that iridescence is a polygenic trait. The iridescent races of these co-mimics form a hybrid zone with non-iridescent races of the same species in the south of Panama, forming two parallel clines. Here I characterise these previously unstudied clines in iridescence, and compare them to the parallel clines of a pattern element controlled by a single locus across the same hybrid zone. This will provide a better understanding of the strength of selection acting on iridescence in *Heliconius*, and the effect of contrasting genetic architectures on the width of these clines.

The genomic basis of ecotype divergence in *Littorina* snails

Anja Westram

University of Sheffield

The marine snail *Littorina saxatilis* contains two morphologically and behaviourally distinct ecotypes, which are adapted to wave exposure and crab predation, respectively. These ecotypes have evolved repeatedly in multiple geographical locations across Europe and show partial reproductive isolation. This system is therefore ideally suited to study the early stages of speciation and the underlying genomic basis. One major question is whether divergently selected loci are shared among instances of parallel divergence, e.g. due to gene flow or repeated selection on shared standing genetic variation. In order to identify loci potentially affected by divergent selection between ecotypes, we performed genome scans, using various marker types and including multiple geographical locations. I will present results indicating that the genomic basis of divergence in this system may be similar among locations on small geographical scales (e.g. within the UK), but largely different on larger geographical scales, suggesting that similar phenotypic changes often evolved independently. While genome scans represent a first step for identifying candidate loci and revealing geographical patterns, the extent of divergent selection acting on outlier loci is often unclear, and their functional role is unknown. I will therefore also briefly present ongoing work on Swedish *Littorina* hybrid zones where we use cline analysis to estimate the strength of divergent selection and apply admixture mapping to establish genotype-phenotype associations.

A computer game to explore the evolution of adaptive variations in mimetic colour patterns

Mónica Arias, David Griffiths, Mathieu Joron , Chris Jiggins, Violaine Llaurens

CNRS UMR7205

The persistence of several warning signals in sympatry is a puzzling evolutionary question, given the positive selection favouring convergence exerted by predators. The evolutionary convergence of complex colour patterns shared by toxic species is shaped by predators' reaction to similar but not identical stimulus, *i.e.* generalisation behaviour. However, the study of generalisation behaviour in complex natural community of predators is challenging, and is thus generally limited to simple variations of prey colour patterns. Here, we used humans as surrogate predators to investigate generalisation behaviours on diverse signals variations displayed in natural populations by the polymorphic butterfly species *Heliconius numata*. The attack behaviour of natural predators' community on the same colour pattern variations was previously tested in a field experiment, allowing comparisons between humans and natural predators' generalisation behaviour. Humans' generalisation capacities were estimated using a computer game simulating a community of toxic and palatable butterflies exhibiting different colour patterns displayed by *H. numata*. In each trial, the same 10 distinct colour patterns were used, and two randomly chosen patterns were associated with a penalty when attacked. Attack rates on the different toxic and palatable colour patterns were recorded, as well as survival time. Phenotypic similarity among the different colour patterns was precisely quantified using a custom algorithm accounting for both colour and pattern variations (CPM method). By analysing attack behaviours of 372 game players, we found that profitable prey gain protection from increased resemblance to unprofitable prey, as previously described for natural predators. Additionally, phenotypic similarity between the two colour patterns associated with toxicity decreased their predation rate, in accordance with Müllerian mimicry expectations whereby shared signals benefit from increased protection. The consistence between our results on humans with the reaction of predators' community on the same variations of colour pattern suggests that our game played by humans is a good proxy of predators' behaviour. This experimental set-up can thus be compared to natural systems, enabling further investigations of generalisation on mimicry evolution.

Polymorphic mimicry as an evolutionary dead-end? Lessons drawn from the genetic architecture of mimetic coloration in Cuckoo finch

V. Laurens and C. Spottiswoode

CNRS UMR7205

Many cuckoo species display mimetic egg colour patterns, preventing their host to distinguish parasitic eggs from their own. Whereas many cuckoo finches display a single egg colour and specialize on a single host species, the African species *Anomalospiza imberbis* exhibit important variations in both egg colour and pattern, and precisely mimics eggs from the polymorphic host species *Prinia subflava*, ranging from turquoise blue to pink colorations, due to biliverdin and protoporphyrin pigments respectively. Although the cuckoo *A. imberbis* seems able to track down most of host egg colour variations, no parasitic eggs exhibits the olive green colour displayed in some host eggs (Spottiswoode & Stevens 2012), which seems to stem from a mix of the two pigments. Here we hypothesized that this may stem from a contrasted genetic architecture of egg coloration in host and parasite: in contrast to the autosomal control of egg colours in the host species, preliminary data suggest that egg coloration in *A. imberbis* may have a maternal inheritance, suggesting a W-linked or mitochondrial location in the control of egg coloration. While the olive green colour displayed by host only may be displayed by heterozygotes at the autosomal locus controlling coloration in host, the expression of this intermediate colour may be prevented in the parasite because the W-location of the locus controlling colour variations. Here we used a theoretical approach to predict the evolution of egg colour frequencies in host and parasites, assuming contrasted genetic architecture of egg coloration and compared the predicted frequencies to field data.

Genetic modularity of mate recognition behaviours and their warning pattern cues

Richard Merrill

University of Cambridge

The genetic architecture of traits that contribute to reproductive isolation can profoundly influence speciation in the face of gene flow. *Heliconius* butterflies are well known for brightly coloured mimetic warning patterns. Because wing colour patterns are also used as mating cues, mimetic shifts can cause both pre- and post-mating isolation. However, shifts in colour pattern cannot drive reproductive isolation alone: they must be accompanied by corresponding mate preferences. We performed a genome-wide QTL analysis of mate recognition behaviours in *Heliconius* butterflies. Our results suggest that at least three QTL of major effect influence mating decisions that contribute to reproductive isolation between the sympatric species *Heliconius cydno* and *H. melpomene*. Of these, one appears to influence attraction to the white wing patterns of *H. cydno*, whilst the other two are associated with attraction to the red patterns of *H. melpomene*. These new results are important for three reasons: First, they represent the only behavioural study in *Heliconius* to consider effects across the entire genome. Second, it is striking that just three loci explain >50% of the difference between the parental species. Finally, because different loci influence the degree of attraction to white females, and others the attraction to red females, these results suggest a modularity underlying visual mate recognition, paralleled by that underlying the corresponding wing pattern cues.

Brains over beauty: *Heliconius* neuroecology

Stephen H. Montgomery

Dept. Genetics, Evolution & Environment, University College London, Gower Street, London, UK, WC1E 6BT

Behavioural and sensory adaptations are often reflected in the differential expansion of brain components. These volumetric differences represent changes in cell number, size, and/or connectivity, which may denote changes in the functional and evolutionary relationships between different brain regions, and between brain composition and behavioural ecology. I will introduce two on-going projects that explore the role of adaptive change in brain composition during the origin and diversification of *Heliconius*. First, I'll discuss the dramatic expansion of the mushroom body, structures involved in learning and memory, and its potential role in *Heliconius* ecology. Second, I'll present evidence that closely related but ecologically divergent species of *Heliconius* have divergent brain architectures. This suggests the adaptive evolution of brain structure and function may play an important role during ecological speciation.

The Ithomiini : the other mimetic butterflies

Marianne Elias¹, Nicolas Chazot², Keith Willmott³, Chris Jiggins⁴, Melanie McClure¹, Doris Gomez⁵ and many others

¹ Institut of Systematics and Evolution of Biodiversity, National Museum of Natural History, Paris, France

² Department of Biology, Lund University, Sweden

³ McGuire Center for Lepidoptera Research, Florida Museum of Natural History, University of Florida, Gainesville FL, USA

⁴ Department of Zoology, Cambridge University, UK

⁵ Center for Functional and Evolutionary Ecology, Montpellier, France

Heliconiine and ithomiine butterflies are two emblematic clades of mimetic butterflies in the Neotropics. While heliconiine butterflies, particularly the genus *Heliconius*, have been extensively studied for development, genomics and selection on wing colour pattern, ithomiine butterflies have mostly been the focus of diversification and community ecology studies. Here, I will present an overview of our research on ithomiine butterflies over the last ten years. We generated a nearly comprehensive phylogeny of ithomiine butterflies (340 species out of 390), which enabled us to decipher the spatial and temporal patterns of diversification of the tribe. The geological dynamics of the Neotropics, especially the Andean uplift, was an important driver of the diversification of ithomiines butterflies by promoting speciation and interchanges between regions, but different clades were affected in different ways. Mimicry was also a likely driver of speciation. On the community ecology side, we demonstrated that co-mimetic species co-occur more often than expected at random at various spatial scales, and that microhabitat and altitudinal niches have undergone adaptive convergence among co-mimics. Mimetic interactions have also affected hostplant use, and we found cases where co-mimics share their hostplant more often, and cases where they share their hostplant less often than expected. We are now zooming in on processes occurring at the population level, and we attempt to test hypotheses about speciation using field experiments and genomic tools.

I will also briefly present a new and exciting collaborative project with physicists and developmental biologists about optics, development and evolution of transparency in Lepidoptera.