

ABSTRACTS

ORAL CONTRIBUTIONS

Roger BUTLIN (invited)

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Adaptation and isolation in winkles and aphids

Speciation may often begin with adaptation to contrasting environments. Progression towards complete reproductive isolation then requires the recruitment of additional traits that are associated with the locally adapted phenotypes and contribute to the total barrier to gene flow. Associations may require the maintenance of linkage disequilibrium in the face of gene flow and recombination but this requirement may be relaxed by 'one-allele' mechanisms or by traits with effects on multiple components of reproductive isolation. Genetic analyses of reproductive barriers should be conducted with these processes in mind.

In the rough periwinkle, *Littorina saxatilis*, shell size and shape show parallel divergence in three European regions. Shell size is both a component of local adaptation and a major determinant of mating pattern. Analysis of multiple genetic markers suggests that demographic separation occurred in parallel in the three regions and also on different shores within regions. However, transcriptome analysis shows that only a small proportion of divergent coding sequences are shared between geographical regions.

In races of the pea aphid, *Acyrtosiphon pisum*, host plant acceptance is critical to both survival and mating pattern. It is determined primarily by chemical cues and so our analysis has focused on candidate chemosensory gene families. A small number of loci, mainly in the odorant receptor family, show strong sequence divergence between races. There are also loci with different copy numbers between races, suggesting another dimension of divergence. The challenge here is to link divergent loci to host plant characteristics.

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Genomics of Introgression across a Species Barrier: Half a Million SNPs across the European House Mouse Hybrid Zone

Understanding speciation as a birth-death process requires study not only of the maintenance of species barriers, but also of those forces acting to break them down ('speciation genes' and 'anti-speciation' genes). We use the European House Mouse hybrid zone as a natural experiment to examine such forces. When SNP marker data is dense on the recombination map it cannot be treated as a series of independent loci but can, instead and more naturally, be analysed in terms of Fisher's representation of the outcome of admixture and recombination: the ancestral source of blocks of DNA along the genome and the position of junctions (arising from recombination) where that source state changes. Cascades in the size of blocks allow ancestral polymorphism to be distinguished from introgression polymorphism. Frequency spectra of introgressed block sizes allow inference of the time since secondary contact. Geographic outlier analysis of blocks allow regions of the genome associated with the introgression of 'anti-speciation' genes across the species barrier to be identified. These insights increase our understanding not only of the mouse species barrier, but also how it can decay in its natural setting.

Astrid T. GROOT (invited)

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Variation in moth sexual communication

In moths, sexual attraction is a strong and well-defined prezygotic isolation barrier: females produce a species-specific sex pheromone that attracts males from a distance. Since in moths female signals and male response are governed by independently assorting genes, it is still a mystery of how moth signal and response may co-evolve. Our research focuses on the identification of these genes to gain insight in the underlying mechanism of divergence, and on the identification of ecological factors that may cause divergence in moth sexual communication. In our quantitative trait locus (QTL) analyses of inter- and intraspecific crosses, we found up to 12 QTL for interspecific pheromone variation and 1-3 QTL for intraspecific variation^{1,2}. We identified homologous chromosomes of our interspecific and intraspecific genetic maps, using RADtags, and found that the same genomic regions are involved in

the inter- to intra-specific variation². In one species (*Heliothis virescens*), we selected for two extreme pheromone types and are currently assessing the consequences of these phenotypes on fitness and male attraction. In two host strains of another species (*Spodoptera frugiperda*), sex pheromone variation seems to hitchhike along with differential timing of mating in the night³. We also found phenotypic plasticity in moth signals: when emerged in the odor of conspecific females, *Heliothis subflexa* females produce a significantly different blend than when emerged in the odor of heterospecific females⁴. Our results will be discussed in light of possible evolutionary forces shaping sexual attraction.

¹Groot et al. 2009. *Insect Biochem Mol Biol* 39: 568-577; ²Groot et al. 2013. *Mol Ecol* 22: 1065-1080 ; ³Groot et al. 2010. *Ecol Entomol* 35 (Suppl. 1): 105-118; ⁴Groot et al. 2010. *J Evol Biol* 23: 2731-2738

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Disentangling the mechanisms of species mate recognition

Mate recognition systems are specific when they lead to homogamous mating. How such systems evolve is an open question that we address here by unravelling the mechanisms of signalling and receiving. We investigated an odour-based species recognition system coupling behavioural, chemical, biochemical and neurophysiological approaches. We chose a model organism on the road to speciation. Two subspecies of the house mouse meet along a secondary contact and interact through a hybrid zone where highly recombined hybrids thrive despite of slightly low fitness compared to the parental types. Populations at the border of the hybrid zone display assortative mate preferences and reproductive character displacement exist between border and allopatric populations of the two subspecies. Mouse urine is an important compartment of species signalling and we analysed its composition comparing border and allopatric populations of the two subspecies. We also applied urine extracts to *ex-vivo* preparations of vomeronasal organs (VNO obtained from transgenic mice) and recorded responses of thousands individual neurons via calcium imaging. Our results indicate strong sexual dimorphism in signalling and that differences in subspecies signalling involve qualitative and quantitative variations of molecules of different nature (urinary proteins, and organic components of different size). The two subspecies appear to share a number of sexual pheromones, although some are present in different concentrations in the two subspecies and could participate to subspecies recognition. The male VNO shows strong segregation of the neuronal responses to female urinary cues of the two subspecies. The nature of the molecules involved in signalling, their pattern of variation and the fact that the vomeronasal organ of laboratory strains discriminate between scents of the two subspecies, strongly suggest that the species recognition divergence process proceeded from pre-existing sensory neurons and molecules.

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Discovery of a recently formed allopolyploid species of *Mimulus* (Phrymaceae) in Scotland provides insights into the early stages of speciation

Hybridisation and polyploidy are thought to be important contributors to the process of speciation in plants. Polyploidy might be particularly influential during the speciation of hybrids as genome doubling results in immediate and strong reproductive isolation between the derivative and parental species. Moreover, polyploidy has also been shown to restore fertility when infertility in hybrids is caused by chromosomal imbalances. Although many plant species have arisen through hybridization coupled with polyploidisation, there are just a handful of examples of recent (<200yrs) allopolyploids.

Here we use a combination of field observations, experimental crosses, and genetic and genome-size analyses to investigate the origin of a new allopolyploid species, *Mimulus peregrinus* (Phrymaceae). The new species arose via polyploidisation of a highly sterile, but widely naturalised hybrid, product of a cross between two clades of American *Mimulus* (*M. guttatus* and *M. luteus*), and introduced to the United Kingdom in the 1800's. We describe the fertility and vegetative performance of parental and derivative taxa, and conduct a field study to assess the frequency and distribution of the new species among other hybrids. Our field and genetic surveys indicate that the allopolyploid *M. peregrinus* has probably originated twice within a small geographic area. We discuss how the co-occurrence of sterile (triploid) and fertile (hexaploid) hybrids in natural populations, as well as the recent origin of the hexaploid, may allow us to disentangle the relative contributions of hybridisation and polyploidy to the genetics and ecology of a nascent species.

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Resource polymorphism and speciation: what can we learn from Northern freshwater fishes?

There is a strong need to better understand what drives the evolution of phenotypic divergence and reproductive isolation. Thus, biological diversity is of key importance today when the world is undergoing major biodiversity changes. In particular we lack knowledge of the integrated role of ecological-, genetic-, plastic- and developmental factors that shape the evolutionary processes behind this diversity.

Extensive recent and repeated divergence of Northern freshwater fishes provides a major opportunity to increase this knowledge. Thus, empirical and theoretical studies focusing on these species have provided evidence of for example: 1) that ecological factors, especially competition, clearly act as agents of natural selection; 2) that there is strong similarity across phylogenetically distinct species, and over vast geographic areas, in the ecological and phenotypic patterns of resource based intraspecific divergence; 3) that there is extensive and dynamic variation in the magnitude of divergence within and among species and over geographic areas; and 4) phenotypic plasticity is commonly identified as playing a significant role in these systems. In the last couple of decades we have seen significant growth in studies of diversity in Northern freshwater fishes. These studies have in most cases focused on individual species and/or localized systems, and often consider one or a few ecological parameters and intrinsic factors. Thus, we have a rather fragmented understanding of the combined role of the ecological factors that facilitate natural selection in these systems, as well as what is the integrated role of genetic-, developmental, maternal- and environmental mechanisms that control or influence the phenotypic differences and levels of reproductive isolation that we observe. Considering that these diverse groups of fishes cover large territories, that their ecosystems are relatively simple, that their geography and colonization history is well known, and that we have the tools to measure and compare their diverse ecological-, reproductive, genetic- and developmental characteristics, they provide major opportunities for future studies to gain new knowledge on the nature of the dynamic ecological and developmental processes that shape biological diversity within and among populations, species and ecosystems.

Ulf DIECKMANN¹ (invited)

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Robust Adaptive Speciation

Adaptive speciation occurs when a population escapes through speciation from remaining trapped at a fitness minimum. Conditions for adaptive speciation naturally arise in a wide variety of eco-evolutionary settings. After reviewing the basics of adaptive-speciation research, this presentation focuses on recent results illuminating and underscoring the robustness of adaptive speciation by examining (a) the implications of different types of competitive interactions, (b) genetic erosion caused by bimodal stabilizing selection, (c) three qualitatively distinct spatiotemporal modes of adaptive speciation, (d) the impacts of habitat boundaries, (e) joint evolution of speciation and dispersal, and (f) the acceleration of adaptive speciation through similar spatial scales for dispersal and mating.

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Genomics of speciation in *Howea* palms

Speciation with gene flow is more common than previously thought, but the mechanisms by which reproductive isolation arise are poorly understood. In plants, sympatric speciation has been demonstrated for the *Howea* palms of Lord Howe Island, where assortative mating occurs due to non-overlapping flowering times, probably initiated by soil preferences. Here we disentangle the genomics underpinning speciation in this system. Combining genome and transcriptome sequencing, including several tissues from multiple individuals of the two species, we provide candidate speciation genes. We present results from a scan for selection of the transcriptome and differential gene expression analyses, and relate the findings to the proposed scenario for speciation in the presence of gene flow. These genomic results are also interpreted in light of several new ecological experiments for the *Howea* palms, thereby providing a comprehensive picture of sympatric speciation in a remote oceanic island.

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Predation drives rapid and repeated sympatric divergence in European whitefish

Repeated formation of intraspecific ecotypes that use different resources has made high latitude lakes and their fish communities important laboratories for speciation research. Resource polymorphism is generally considered to occur in species-poor systems in response to intense intra-specific competition and high ecological opportunity. Recent findings however, suggest links between polymorphism and certain species combinations or complex fish communities, a fact that raises questions about the generality of this paradigm. Here we show that predation can induce divergent life history strategies on an extremely short timescale. Data from 351 Scandinavian lakes show that presence of a large piscivore, the northern pike (*Esox lucius*), induces dwarfs, giants or divergence into both ecotypes in populations of European whitefish (*Coregonus lavaretus*) depending on lake characteristics. Using 76 whitefish introductions made between year 1784 and 2009 as natural experiments, we found that 50% of populations exposed to predation from pike had formed distinct ecotypes in 72 years. This demonstrates that divergence into the frequently observed "species pairs" of dwarfs and giants can be much faster than what is currently recognized. Our findings emphasize the importance of understanding direct and indirect implications of size-specific mortality rates for imposing divergent ecological selection along the benthic-pelagic resource axis. Moreover, they illustrate the potential importance of predation as a driving force behind the creation and maintenance of biodiversity in nature.

Leonie C. MOYLE (invited)

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The evolutionary timing and pattern of accumulation of reproductive isolation loci

Data from genetic mapping studies between species, along with information on evolutionary relationships among them, can be used to reconstruct the timing of the underlying causal changes contributing to species differences. However to do so requires information evaluating whether QTL (Quantitative Trait Loci) that are chromosomally colocalized between >1 species pair are due to homologous alleles. Here we use cross-specific complementation tests to evaluate evidence for allelism at reproductive isolation QTL that are chromosomally colocalized in >1 species cross between three plant (*Solanum*) species. With these data, we assign all known isolation loci among these species to branches of their phylogeny. We find that: First, there is no evidence that loci that accumulate early have larger effect sizes. Second, the majority (80-90%) of isolation loci arise later in the history of divergence between species; that is, most isolation loci are clustered on recent evolutionary branches. Third, for pollen sterility QTL, this pattern is indistinguishable from a 'snowballing' accumulation of isolation loci, as predicted by theory.

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Niche dimensionality and the genetics of ecological speciation

The properties of the adaptation process in populations evolving in allopatry affect the fitness of their hybrids upon secondary contact, and the initiation of speciation by post-zygotic reproductive isolation. In particular, it has recently been suggested that the number of ecological axes along which populations diverge plays a crucial role in ecological speciation, but formal theoretical predictions are lacking for this idea. I will present results of an adaptive landscape model that addresses this question, based on Fisher's geometrical model of adaptation. A central aspect of this model is that reproductive isolation results from the phenotypic effects of mutations that get fixed in each parental population during adaptation, and depends on the environment. Under multivariate stabilizing selection, the mean fitness of hybrids is reduced by the segregation variance - as exemplified by extreme transgressive phenotypes -, causing recombinant genotypes to deviate from the optimum across environments, which offers a reinterpretation of Dobzhansky-Muller incompatibilities in an ecological context. Reproductive isolation depends on the evolutionary trajectories of adapting populations, rather than on their final divergence. We use this framework to find how mean hybrid fitness depends on the dimensionality of the adaptive landscape, under (i) adaptation to different optima, or to the same new optimum; and (ii) divergence at mutation-selection-drift equilibrium around a constant optimum. The latter generates a pattern of temporal accumulation of reproductive isolation that matches observation from numerous taxa.

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Symbiont-triggered speciation: concepts, causes and consequences

Current standard models of speciation disregard potential impacts of microbial reproductive tract symbionts fostering host speciation. Based on numerous theoretical models but limited experimental data, prime candidates driving symbiotic speciation in arthropods are intracellular bacteria belonging to the genus *Wolbachia*. They are inherited symbionts of many arthropods capable of manipulating host reproductive biology for their own benefits. However, it is an ongoing debate as to whether or not reproductive symbionts are capable of triggering host speciation in nature and if so, to what extent. Hence we applied the classic model system of symbiotic speciation manifest in neotropical *Drosophila paulistorum* semispecies by means of present-day molecular approaches and artificial symbiont-depletion experiments. This superspecies, consisting of at least six reproductively isolated semispecies, has been the object of attention of an array of researchers since at least 1955, when it was initially trapped mid-evolution in flagrant *statu nascendi*. In this system it has been proposed that microbial endosymbionts foster incipient speciation.

We have recently demonstrated the α -proteobacteria *Wolbachia* as the maternally transmitted core endosymbiont of *D. paulistorum* semispecies that presently foster symbiotic speciation by triggering pre- and postmating isolation mechanisms. Here we will report on (i) the impact of *Wolbachia* on driving artificial *de novo* reproductive isolation in *D. paulistorum*, (ii) their effects on host pheromone expression patterns and sexual behaviour, and (iii) unorthodox transmission modes of mitochondria in hybrids. Finally we will introduce a second symbiotic speciation system in *statu nascendi*, i.e., the *Glossina morsitans* species group of tsetse flies, where conspecific but incompatible *Wolbachia* infections trigger postmating isolation in nature.

Jenny W. BOUGHMAN (invited)

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Speciation by divergent sexual selection

Whether sexual selection leads to speciation is controversial. Theoretically, sexual selection should promote speciation because it leads to rapid evolution of mating traits. Several elegant case studies offer support. In contrast, comparative studies are equivocal, casting doubt on the general importance of sexual selection to speciation. We show the discrepancy exists because comparative studies ignore the very reason sexual selection causes speciation – because it causes divergence in the traits used to select mates and ensure fertilization, thereby preventing mating between species. Former comparative studies have focused instead on the strength of sexual selection. We test two fundamental predictions in a comparative context: how sexual selection through female choice affects both divergence in male display traits and the accumulation of reproductive isolation. First, we test whether the strength of mate preferences or the extent to which they differ between species predict the amount of diversification in male display traits. We use quantitative measures of mate preference and display divergence from case studies across a diverse set of taxa. We find that diversification in male display is better explained by preference divergence rather than preference strength. Next, we test whether the strength or divergence of sexual selection cause elevated levels of reproductive isolation. We use comparative data for birds, and find that sexual selection contributes importantly to reproductive isolation in birds, but only when it generates differences in mating traits. As divergence in sexually selected display traits increases, significant reproductive isolation accumulates. Our results highlight that the importance of sexual selection to speciation arises when it differs between species. Without such divergence, even when sexual selection is strong, little reproductive isolation results. Thus, the distinction between how strong sexual selection is compared to how much it differs between populations is crucial to understanding the role sexual selection plays in speciation. The evolutionary consequences of strong sexual selection might be rapid evolutionary change within a lineage, whereas divergent sexual selection is likely to produce differences between lineages in traits that mediate mating and fertilization.

Jochen WOLF (invited)

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Linking genes and 'magic traits': Narrow islands of genomic divergence across the hooded and carrion crow hybrid zone

Unravelling the mechanisms of genome divergence between young evolutionary lineages is key to understanding the origin of species. The European crow hybrid zone between all-black carrion crows and grey-coated hooded crows is a textbook example of incipient speciation where pre-zygotic isolation through sexual selection is believed to play a

significant role. We assembled and annotated the 1.05 Gb genome of one hooded crow individual, re-sequenced 60 genomes from two populations of each taxon, investigated gene expression profiles under common garden conditions and conducted qualitative immunohistochemical assays on growing feather follicles. Across a background of low genome-wide differentiation ($F_{st} \sim 0.02$), we find evidence for only a handful of larger divergence peaks (size $\sim 1\text{Mb}$). These regions as well as smaller divergence runs of only a few SNPs are associated with differentially expressed candidate genes from within the melanogenesis pathway. This study elucidates the genetic basis of colour differences between the two taxa and provides evidence that few genes of major effect associated with a sexually selected trait can significantly contribute to reproductive isolation.

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Three modes of adaptive speciation in spatially structured populations

Adaptive speciation with gene flow via the evolution of assortative mating has classically been studied in one of two different scenarios. First, speciation can occur if frequency-dependent competition in sympatry induces disruptive selection, leading to indirect selection for mating with similar phenotypes. Second, if a subpopulation is locally adapted to a specific environment, there is indirect selection against hybridizing with maladapted immigrants. While both of these mechanisms have been modeled many times, the literature lacks models that allow direct comparisons between them. In our study, we incorporate both frequency-dependent competition and local adaptation into a single model, and we investigate whether and how they interact in driving speciation. We find that a combination of spatial heterogeneity and weak within-deme frequency dependence leads to a new speciation mode, where speciation is initiated by selection against maladapted migrants, but completed by within-deme competition in a distinct second phase. While this mode has some similarities to classical reinforcement, it happens considerably faster, and both newly formed species go on to coexist in sympatry.

Mark KIRKPATRICK (invited)

Section of Integrative Biology, University of Texas at Austin, USA

The evolution of pre- and postzygotic isolation

In the first part of the talk I describe a meta analysis of assortative mating in animals. In a review of 1400 studies, we find that moderately strong ($r = 0.2$) assortment is common. Surprisingly, there is no evidence of negative assortment. I discuss the implications for speciation, in particular the assumption common to many models of speciation that the strength of assortment evolves adaptively. In the second part of the talk, I describe our study of a genetic incompatibility in fish of the genus *Xiphophorus*. In the first analysis of the coevolution of genes involved in a Dobzhansky-Muller incompatibility, we find evidence that copy number variation (presence or absence) of an oncogene evolves in a correlated fashion with the promoter of a putative melanoma repressor.

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Investigating the role of a Robertsonian fusion in divergence and speciation

Gene flow and recombination directly oppose divergence and speciation by homogenizing population/species specific gene combinations that confer ecological adaptations and/or reproductive isolation. Theory suggests that chromosomal rearrangements may play a critical role in speciation by reducing recombination in parts of the genome. Such a situation could allow for increased divergence and the evolution of reproductive isolation by preserving species specific gene complexes in the face of gene flow. Recent evidence has linked certain types of chromosomal rearrangements (inversions) and the development of reproductive isolation. However, other types of common rearrangements, such as chromosomal fusions, have yet to be investigated in this capacity. Here we will determine the importance of a chromosomal fusion to the development of reproductive isolation and ecological divergence in two killifish species in the genus *Lucania*. These species are sympatric and there is evidence for gene flow. Using backcrosses we map QTLs involved in ecological divergence (salinity tolerance), behavioral isolation (male mate choice), and intrinsic isolation (male sterility). The location of these QTLs will elucidate the role of recombination in speciation and divergence with gene flow in *Lucania*.

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Are Robertsonian fusions barriers to introgression in a house mouse hybrid zone?

Genomic regions of low recombination are expected to contribute to the accumulation of incompatibilities allowing speciation with gene flow to occur. While chromosomal inversions are well documented in such processes, the role of Robertsonian (Rb) fusions (that join two acrocentric chromosomes by their centromere to form a metacentric one) is less clear. The house mouse is an emblematic model of Rb variation since one subspecies, *M. m. domesticus*, displays >80 Rb races. Previous cytogenetic studies demonstrated a decrease in recombination rates along the proximal 50% of Rb chromosomes in both homozygotes and heterozygotes. This study explores the role of Rb fusions in reducing gene flow by investigating their effect on genome admixture between two subspecies of the house mouse. In Denmark where the two taxa form a tension zone, *M. m. domesticus* carries 3 Rb fusions while *M. m. musculus* has an all-acrocentric karyotype. Genome admixture was assessed using 127 SNPs diagnostic of the two subspecies and covering the proximal 25% of 3 chromosomal arms involved in Rb fusions and 2 acrocentrics. We compared patterns of genomic admixture in the proximal regions to those at 86 diagnostic loci randomly distributed in the genome by fitting a genomic cline model with 3 parameters (centre and slope in each subspecific context). In the *domesticus* genomic context, Rb clines were much steeper than that of the average reference loci. Their centre was displaced in favour of *musculus*, however, due to introgression of large chromosomal blocks, and both of these patterns were stronger for the most proximal loci. This is compatible with the predicted effects of reduced recombination in Rb fusions coupled with incompatibilities in these genomic regions. In addition, clines for acrocentrics were smoother than average in the *musculus* genomic context, suggesting that other undescribed effects are acting, such as a possible advantage of *domesticus* acrocentric centromeres.

Mathieu JORON

UMR CNRS 7205, Muséum National d'Histoire Naturelle, Paris

Inversions, supergenes and the build-up of linkage disequilibrium between multiple traits

Chromosomal inversions are well-known for their suppressing effect on recombination, and can lock together multiple beneficial gene variants controlling specific trait combinations. There are historical examples, as well as numerous new cases of structural variation associated with the maintenance of well-differentiated morphotypes, ecotypes, or species in sympatry in diverse taxa. Chromosomal rearrangements are therefore important mechanisms of genomic architecture evolution, which merge the control of multiple beneficial traits under a simple inheritance. Thus they provide a way of building up loci of large phenotypic effect, often found to be associated with adaptive variation and radiations. But exactly how multiple beneficial traits become recruited within inversions at the population level is still unclear. In this talk, I will discuss the origins of rearrangement-associated adaptations from plant and animal taxa. I will highlight the role of the ecology of each individual trait and their different combinations, the role of the selection regimes underlying the fitness benefits of tight linkage, and the role of introgression across the species boundary. A continuum of genomic architectures underlies adaptive variation from stable polymorphisms within populations to ecotypes and to ecological speciation. I will take inversions and supergenes as excellent genomic microcosms to improve our understanding of the process of adaptation and the build-up of linkage disequilibrium between multiple traits.

Patrik NOSIL (invited)

Animal and Plant Sciences, University of Sheffield, UK

Experimental evidence for rapid and genome wide evolution during ecological speciation

The ability of populations to adapt to novel ecological conditions affects the persistence and origin of species. However, stochastic processes such as genetic drift, limited genetic variation, homogenizing gene flow, and shifting natural selection can hamper adaptation and increase extinction risk, making predicting evolution in response to ecological challenges difficult. Here, we combine a manipulative field experiment, whole genome sequencing, and population genomic analyses of experimental and natural populations to show predictable effects of natural selection on genome evolution across timescales. In a release and recapture field experiment we transplanted stick insects to novel host-plant and climatic environments. Using Bayesian models applied to 186,576 loci we found substantial and genome wide allele frequency changes caused by mortality between release and recapture in the experiment. Comparisons of observed changes to null models of random mortality indicated that although most changes were consistent with drift, natural selection acting on standing genetic variation affected numerous loci distributed across the genome. Evidence for selection was strongest for populations transplanted to more novel environments. Consistent with a pervasive effect of selection on genome evolution, the extent to which selection affected loci in the experiment was correlated with allele frequency differences among long-established natural populations, especially

those experiencing low gene flow. The findings illustrate dual effects of gene flow on adaptation, constraining divergence between populations but maintaining genetic variation that allows for rapid responses to natural selection induced by environmental change.

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Hybrid speciation driven by discordant cline movement of mito-nuclear and sex-linked incompatibilities

It is commonly thought that speciation by hybridization differs radically from more typical non-hybrid speciation, where sex-linked genes and mito-nuclear incompatibilities influence reproductive isolation disproportionately. In contrast, special, transgressive hybrid phenotypes or genome organizations are thought to be required for hybrid species to escape gene flow from their parents. Studying a recently recognized hybrid bird species, the Italian sparrow *Passer italiae*, we test the novel hypothesis that a hybrid taxon can inherit the reproductive barriers that normally separate its parent species through recombination and discordant cline movement. We identified an unbiased set of putatively parent species-diagnostic SNP markers through whole-transcriptome sequencing of six individuals of each of the two parent species. After filtering for coverage, genotyping success (>97%) and multiple SNPs per gene, we retained 86 species-informative, genic, nuclear and mitochondrial SNP markers from 84 genes for analysis of 612 male individuals. We show that the Italian sparrow is isolated from both its parent species through discordant cline movement of mitochondria and blocks of sex-linked genes to the hybrid-parent range boundaries. Genomic conflict via interactions between mitochondria and sex-linked genes with mitochondrial function5 (“Mother’s curse”) at one boundary and centromeric drive at the other best explain our findings. This mechanism of discordant cline movement of reproductive isolation genes depends on the dissociation of as few as two different parental species’ isolating mechanisms to create species boundaries between the hybrid and both parent species. Consequently, speciation by hybridization may be more likely than previously suspected, calling for a re-evaluation of the role of hybridization in diversification.

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Hybridization promotes colonization of new environments

Transgressive segregation, the expression of traits in hybrids that are absent from parental populations, could promote hybrid speciation. Although broadly accepted, the concept remains largely untested experimentally. We crossed 31 phenotypically and genetically diverse yeast strains (*Saccharomyces cerevisiae* and *S. paradoxus*), and compared the growth of the parental strains, the F1, and the F2 hybrid swarms in seven environmental clines, each of which extended beyond the range permitting growth of the parent strains. We made multivariate phenotypic fingerprints of the hybrids from each cross to quantify the extent of hybrid transgression. We found transgression was frequent, with 20% of all hybrid crosses able to grow under conditions too extreme for the parents. We also found that hybrids outcompeted their parents under direct competition in a common habitat. Transgression in hybrids between distant relatives was stronger in ecologically relevant environments, where resource use and resistance are likely polygenic. These results suggest that transgression in ecophysiological traits can generate vast adaptive variation and functional novelty, opening up new evolutionary trajectories for hybrids, and facilitating the colonization of previously inaccessible peaks in an adaptive fitness landscape. Our results also suggest that hybrid speciation is most likely to occur at the peripheries of species ranges, where genetic and ecological opportunities co-exist.

James MALLET (invited)

Harvard University (Department of Organismic & Evolutionary Biology) and University College London (Department of Genetics, Evolution and Environment)

Promiscuous genome exchange among hybridizing *Heliconius* butterflies

It is a seductive idea that species are independent evolutionary units. Natural hybridization is rare in nature on a per-individual basis, but it may affect many species. Brightly-colored *Heliconius* butterflies engage in Müllerian mimicry of other species. Most of this mimicry is due to adaptive reconstruction of similar patterns, but we’ve long suspected that colour patterns are exchanged among some of the more closely related species that hybridize occasionally in the wild. We have recently demonstrated gene flow on a genome-wide basis among such species. Furthermore, regions that determine mimicry have indeed been exchanged repeatedly among closely related species to form new,

adaptive combinations. Through combined effects on mating behaviour and signalling to predators, these novel colour patterns have also been involved in triggering evolution of new species. While this may seem unusual, the genomic evidence we've revealed is among the first data suitable for testing these possibilities. I predict that hybridization and homologous recombination among species is much more important than hitherto recognized.

Géza MESZENA¹

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Three-phase transition to reproductive isolation

Adaptive emergence of reproductive isolation is studied in a multilocus model of sympatric speciation modified from Dieckmann & Doebeli (1999) with the aim of deeper understanding of genetic mechanisms. A three-phase transition to clean eventual reproductive isolation was observed in a significant fraction of the parameter space with platykurtic carrying capacity. First, partial assortativity emerges, expanding population variance to be compatible with the width of resource distribution. Second, genetic variance erodes away and an increasingly bimodal phenotype distribution emerges in a slow process. Third, a fast transition occurs into complete separation of the two emerging species. The first phase is driven by disruptive ecological selection. The time-consuming second phase is a self-accelerating process due to (ecological and sexual) optimizing selection in the two, increasingly isolated subpopulations. The fast third phase is driven by run-away sexual selection. The incomplete separation of species, that is characteristic to the slow phase, is probably common in nature. If the final stage is reached, the two populations become cleanly segregated, but a detectable genetic footprint of the intermediate phase should remain.

Paris MARGOT

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Speciation in wild tomatoes: inference from genome-wide sequence data

Due to their fairly recent divergence, differences in mating system, and the genomic resources available, wild tomatoes (*Solanum* section *Lycopersicon*) offer a powerful model to study speciation at a genome-wide scale. Our study aims to characterize and compare the distribution of intraspecific nucleotide polymorphism and interspecific nucleotide divergence between several closely related wild tomato species. Furthermore, we focus on understanding the relative importance of various evolutionary and ecological processes that can promote or prevent genomic islands of differentiation, such as differences in mating system and levels of recombination, varying strength of reproductive isolation, purifying or positive selection, and demographic/speciation history. Using individual tagging and the Illumina Hi-Seq 2000 platform, we have sequenced 106 transcriptomes, using flower buds as the source of mRNA. Most of these data represent species-wide samples of seven green-fruited species, two of them with a highly selfing mating system. First, based on 4350 genes that are scattered along all 12 chromosomes, we have characterized the genomic distribution of levels of intraspecific polymorphism and divergence between our 21 species pairs. Subsequently, these data will be used to assess the impact of differences in the strength of natural selection (e.g. as a consequence of differences in species' effective population size) and reproductive barriers, as well as differences in levels of effective recombination on patterns of genomic divergence.

Martine MAAN (invited)

Centre of Behaviour and Neurosciences, University of Groningen, the Netherlands

Divergent ecological selection on mating traits: alternative mechanisms and their consequences for speciation

Divergent selection on sexual communication traits may promote reproductive isolation, but may also lead to geographic variation without speciation. In particular, when sexual signals diverge between populations but sexual preferences do not, speciation may be slow or not happen at all. In contrast, divergent ecological selection on preferences can lead to divergence in signals as well, potentially providing a more powerful mechanism for speciation. Sensory drive, i.e. the adaptation of communication traits to their sensory environment, can affect both senders and receivers of sexual signals. We investigate how these effects may contribute to speciation in the cichlid fishes of Lake Victoria. These fish inhabit diverse underwater light environments, and closely related species often differ in sexually selected coloration. We ask how divergent adaptation to different light environments affects male colours as well as female colour preferences, to establish whether cichlid colour vision could act as a 'magic trait' in speciation: subject to divergent ecological selection and at the same time pleiotropically determining female mating preferences.

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The geography of introgression in a patchy environment and the misperception of fast speciation

When incompletely isolated taxa coexist in a patchy environment (e.g. mosaic hybrid zones, host races), patterns of variation may differ between selected traits/genes and neutral markers. While the spatial structure of selected traits/loci tends to coincide with habitat variables (Genetic-Environment Association or GEA), genetic differentiation at neutral loci rather depends on geographic distance (Isolation-By-Distance or IBD) at a large scale, although revealing GEA at a small scale. This discrepancy is taken as an evidence for parallel primary divergence driven by local adaptation (*i.e.* recent or ongoing ecological speciation). An alternative hypothesis, often hastily ruled out, is that speciation was allopatric, followed by a secondary contact, and that introgression has subsequently erased the signal of past differentiation at neutral loci. We present a model of neutral introgression in a mosaic hybrid zone and describe how GEAs dissipate with time and how the neutral variation self-organizes according to the environment and geographic structures. We show that although neutral loci can be affected by environmental selection, they are often more affected by history and distance: the neutral structure retains the initial geographic separation more than it correlates with the environment during the colonization and introgression phases, and it converges to a migration-drift balance, the most frequent outcome of which is GEA at a local scale but IBD at a large scale. This is the exact pattern usually attributed to recent parallel ecological speciation. We argue that the history of speciation should ideally be reconstructed with selected loci, not amnesic neutral markers. Once done, unfortunately, adaptation from standing genetic variation has been put forward to maintain the initial hypothesis that speciation was recent while spatial reorganization of old co-adapted genetic backgrounds is equally likely.

Richard BAILEY

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The role of genetic constraints during speciation: Hybridization and the G matrix.

Adaptation and hence adaptive divergence is affected by the shape of the G matrix - the pattern of genetic correlations between traits - which constrains evolution in phenotype space to be more likely to follow particular 'lines of least resistance' or trajectories of high 'evolvability'. But while progress is being made in studying G matrix evolution within species, little is known about how such constraints evolve during hybridization between divergent genotypes, and hence how they influence (1) isolation between hybridizing species, (2) the potential for local adaptation in hybrids and hybrid zones, and (3) hybrid speciation. How does gene flow affect the orientation of evolvability in hybrids? Would the mixing of two genomes increase or decrease dimensionality of available genetic and phenotypic variation? How would the pattern of constraints in hybrids affect gene flow between the parent species and hence future divergence? Do changing constraints in hybrids influence the likelihood of hybrid speciation and of local adaptation in a hybrid species? To answer these and related questions, we examine patterns of trait covariance in house and Spanish sparrows, their homoploid hybrid descendant species the Italian sparrow, and in a narrow hybrid zone between Italian sparrows and their parent species the house sparrow. Through examination of patterns of phenotypic constraints (the P matrix) we ask to what extent hybridization and continuing gene flow facilitate or interfere with local adaptation in the hybrid species and hybrid-parent hybrid zone.

Reto BURRI¹

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The landscape of genome divergence in *Ficedula* flycatchers

Unravelling the landscapes of genome divergence between individuals, populations, and species is key to understand the genomic architecture of biological diversification, and the forces driving its evolution. In the present study we sequenced the collared flycatcher (*Ficedula albicollis*) genome, and studied the genomic divergence between the naturally hybridizing but reproductively isolated collared flycatcher and pied flycatcher (*F. hypoleuca*). Population genomic analysis of each 10 genomes of both species reveals a highly heterogeneous landscape of genome divergence with about 50 'divergence islands' that show ~50-fold higher differentiation than the genomic background. The non-randomly distributed divergence islands are characterized by reduced nucleotide diversity, skewed allele-frequency spectra, elevated linkage disequilibrium and reduced proportions of shared polymorphisms in both species, in line with parallel episodes of selection. The high incidence of divergence islands with genomic regions resistant to sequence assembly, potentially including centromeres and telomeres, indicate that complex repeat structures may drive species divergence. In order to identify the molecular and evolutionary forces driving the evolution of divergence islands in *Ficedula* flycatchers, these insights are complemented with population genomic analyses of an additional 200 flycatcher genomes from multiple populations and species.

Axel MEYER (invited)

Faculty of Biology, University of Konstanz, Germany

Incipient speciation and parallel evolution in adaptive radiations of Nicaraguan crater lake cichlid fish

In a setting of a natural experiment in several young (about 1200 to 22000 year old) crater lakes in Nicaragua small adaptive radiations of cichlid fish (of the *Amphilophus citrinellus* species complex) have formed. So far up to six endemic species have been described for each of these species flocks that are all derived from an ancestral population in the two large lakes in Nicaragua. Some features such as limnetic-benthic phenotypes, thick-lipped forms, and papilliform-molariform dentition have evolved repeatedly. Also, several of these species are polychromatic as an amelanistic "gold" form is found, in many, but not all, of these crater lake radiations. Through a number of complementary ecological, evolutionary, genomic approaches including population genomics, QTL analyses and a de novo complete genome project, we are studying this system to better understand the processes of sympatric ecological speciation and the repeated evolution of adaptive traits.

Christophe DESTOMBE¹

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Unraveling sibling species within *Pylaiella littoralis* (Ectocarpales, Phaeophyceae) using phylogenetic, ecological and biological evidences

The wide phenotypic variability and the lack of diagnostic characters in the genus of the filamentous brown algae *Pylaiella* render the systematic study of this genus problematic. During the last century, many authors have described a multitude of species, varieties or forms according to their morphology, their life history and/or their ecology, nevertheless no clear consensus have been reached. In the present study, we investigated the diversity of *Pylaiella littoralis* along the Brittany coast using a DNA barcoding multilocus approach with nuclear, mitochondria and chloroplast markers combined with population genetics approach to study the reproductive systems using microsatellite markers. Moreover, spatio-temporal sampling was conducted to detect genetic variations among populations as well as possible phenological difference between populations. We sampled 180 individuals from 8 sites located along the Brittany coast between Saint-Malo and Quiberon (450km) from march to october. Our results highlighted the occurrence of two genetic entities based on mitochondrial sequences showing a minimum of 2.2% divergence between them. Instead the analysis of chloroplast and nuclear sequences revealed a single group (haplotypic divergence ranging from 0 to 0.7% and 0 to 0.47% respectively) which may reflect ancestral genotypes corresponding to a recent differentiation of the two entities. Analyze of the geographical clustering pattern based on microsatellites revealed a high genetic differentiation between two entities and also among populations of each entity. These two species were found in the same environment; however their growths were shifted in time according to season. This phenological shift provides an ideal framework for exploring questions concerning adaptive divergence as possible mechanisms of speciation.

Philine G. D. FEULNER¹

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Genome wide patterns of divergence: a comparison of five parapatric lake-river population pairs of three-spined sticklebacks

In the last years, the accessibility of genetic data has enabled the study and comparison of whole genomes at a population level, revealing the highly dynamical properties of genomes. Most of these findings focus on genetic model organisms wherein the impact of ecological context is often overlooked. Here, we characterize the differential genome evolution across 10 natural populations of three-spined sticklebacks, a fish species that has recently colonized various habitats and has since undergone substantial and recurrent phenotypic divergence. Using 60 whole genome sequences (15x coverage each) from geographically different lake-river population pairs, we identify genomic regions of divergence between these distinct ecotypes. We identify different genes and genomic regions potentially associated with ecological differentiation in the five lake-river comparisons. This suggests that divergence patterns between lakes and rivers can involve multiple molecular pathways. Interestingly, we find some associations between genomic structural variations and regions of pronounced divergence. In studying these natural populations undergoing recent ecological differentiation, our findings broaden our understanding of the molecular underpinnings of recurrent ecological adaptation and offer perspective on the genomics during the early stages of divergence.

Markus PFENNINGER¹

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Convergent evolution of *cox*-genes in H₂S-tolerant fish: a key adaptation drives ecological speciation

Colonisation of extreme environments requires key adaptations that allow their exploitation. Replicated niche shifts offer a unique opportunity to study such key adaptations, especially when *a priori* predictions can be formulated as to which genes should be involved. Populations in the *Poecilia mexicana*-complex have repeatedly colonised springs containing volcanic hydrogen sulphide (H₂S). H₂S blocks the cytochrome c oxidase-complex (COX), so we predicted their decreased H₂S-susceptibility to evolve in sulphide ecotypes. Using enzyme activity assays under different H₂S-concentrations, mitochondrial genome sequencing, protein structure modelling, and population genetic approaches we demonstrate that decreased H₂S-susceptibility evolved in parallel in two sulphide lineages. Convergence extended to the molecular level, as evidenced by shared amino acid substitutions in the *cox1* and *cox3* genes. We hypothesise one of these substitutions to trigger conformational changes in COX1 that block the H₂S access. In a third sulphide population we detected no decreased H₂S-susceptibility of COX, suggesting that H₂S resistance is achieved through another mechanism. Sulphide ecotypes are reproductively isolated from clear water populations, and convergent evolution of *cox*-genes may be a key component of ecological speciation in this complex. Our study also demonstrates that even closely related lineages may follow different molecular evolutionary paths of adaptation in response to the same selection pressure.

Åke BRANNSTROM¹ (invited)

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Eco-evolutionary modeling of diversification and speciation

Many studies in speciation research assume that ecological conditions for diversification exist and investigate mechanisms that can overcome the cohesive nature of sexual selection. In this presentation, I will emphasize the other side of the story and demonstrate how modern theoretical tools, in particular adaptive dynamics, can be used to elucidate and uncover the ecological conditions that promote diversification and speciation. Several case studies will highlight the role of ecological interactions and population structure in diversification and demonstrate the untapped potential of eco-evolutionary modeling to improve understanding of the processes that facilitate diversification and speciation in specific systems.

Anne-Sophie BONNET-LEBRUN¹

Co-authors: Andrea Manica², Anders Eriksson² and Ana Rodrigues¹

The temporal dynamics of phylogenetic tree shape in neutral community models

The shape of phylogenetic trees reflects past speciation and extinction dynamics. Measures of tree shape have thus received considerable attention as indicators of past macroevolutionary processes. Two types of measures have received particular attention, quantifying tree balance (e.g., β ; Blum & François 2006) or the distribution of branch lengths to capture the “tempo” of diversification (γ ; Pybus & Harvey 2000). Artificial trees generated from models can shed light on the evolutionary processes underpinning real phylogenies. Models where lineages evolve through birth-death processes are commonly used, but these fail to reproduce the typical imbalance observed in empirical trees (Mooers 1997) or their negative γ values (presumed to reflect a slowdown in diversification rates; Nee 2007). This may be explained by the assumptions in these models that lineages evolve independently and that speciation/extinction rates are the same across lineages. More recently, neutral community models have been proposed as more suitable null models, as they take into account the competitive interaction between species and variation in probabilities of speciation/extinction with lineage abundance (Davies et al. 2011). Previous results found that although neutral models can predict the levels of imbalance in real trees, they still fail to reproduce the negative γ s. Here, we investigate how the shape of simulated phylogenetic trees evolves over time in neutral communities, whereby a single lineage evolves from an initial colonisation event from a remote source into a spatially-structured region. We found that the γ of these simulated trees varies widely over time, with values in young trees approaching those of empirical trees (negative γ s), but becoming progressively higher (and positive) even beyond the time when trees reach demographic and evolutionary equilibrium. This suggests that an explanation for the low γ values in empirical phylogenetic trees could be that they have not yet reached equilibrium.

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Extraordinarily rapid speciation on oceanic islands: the case of *Cheirolophus* in Macaronesia

Volcanic islands are excellent natural laboratories to test speciation and diversification hypotheses. For this reason, in the last decades the Macaronesian archipelagos have become a key subject for several evolutionary studies in many diverse plant groups. Particularly, molecular analyses have been significantly useful in addressing some major evolutionary topics on island radiation processes: (1) allopatric speciation; (2) adaptive radiation; (3) interspecific hybridisation. The genus *Cheirolophus* (Asteraceae), comprising c. 20 endemic species to the Canary and Madeira archipelagos, represents an ideal study case for discussing these issues. Here, we employed the latest Bayesian-based phylogenetic tools to analyse *Cheirolophus* radiation processes, not only in a reliable timescale but also incorporating spatial biogeographic data. Our results confirmed the monophyly of Macaronesian taxa and suggested that radiation within insular taxa started in the last 2 My. Showing an exceptionally high diversification rate (4.17-0.34 species/million years), the case here presented could be considered as one of the most remarkable examples of explosive plant radiation in oceanic islands so far reported. Certainly, the diversification of the genus in Macaronesia would be better interpreted as the result of coupled allopatric speciation (intra- and inter-island) and incipient adaptive radiation. To help explaining the explosive radiation of *Cheirolophus* within such a small area, some interrelated factors of particular importance were considered, such as island and habitat characteristics, plant features (i.e. dispersal syndrome, reproductive traits) and Pleistocene climatic changes.

Robin HOPKINS (invited)

Section of Integrative Biology, University of Texas at Austin, USA

Selection and speciation: From genotype to phenotype to reproductive isolation

A major goal in evolutionary biology is to understand the role of natural selection during the process of species formation. Specifically, there is interest in identifying the genetic basis of traits causing reproductive isolation, and measuring the strength and mechanisms of selection on these traits. My research investigates how natural selection contributes to the evolution of reproductive isolation through the study of reinforcement. Reinforcement is the process in which selection against hybridization favors the evolution of reproductive isolation between emerging species. Flower color variation in the wildflower *Phlox drummondii* was one of the first investigated examples of reinforcement in plants. Both *Phlox drummondii* and *Phlox cuspidata* produce similar light-blue flowers throughout the allopatric

parts of their ranges. However, in the area of sympatry *P. drummondii* has dark-red flowers, which decreases hybridization between the two species. The color transition is caused by cis-regulatory mutations in two genes involved in the production of floral anthocyanin pigments. I will report results from common-garden field experiments showing strong selection acting on genetic variation at both flower color loci. In sympatric populations I demonstrate the operation of reinforcing selection and quantify the strength of that selection under natural conditions. I find that a single allele at a flower-color gene substantially decreases hybridization between the two *Phlox* species. Pollinator constancy based on flower color causes this increase in reproductive isolation. In allopatric populations I find selection favoring the ancestral light-blue flower color. My experiments suggest strong pollinator preference is likely responsible for maintaining the light-blue flower color in *P. drummondii* populations without *P. cuspidata*. By combining genetic investigations with field-based experiments I am able to better understand the role of natural selection in the evolution of reproductive isolation during the process of reinforcement.

Pim EDELAAR

Department of Molecular Biology and Biochemical Engineering, University Pablo de Olavide, Sevilla, Spain

Matching Habitat Choice as a driver of speciation

We know that habitat choice genes can assist speciation in e.g. phytophagous insects. However, habitat choice can also create assortative mating if individuals choose the habitat type in which they perform best based on their individual phenotype (what we previously named /Matching Habitat Choice/). This could kick-start a process of divergence or even could create substantial reproductive isolation by itself. Since there are no alleles for habitat choice segregating, this could be viewed as a one-allele speciation mechanism, avoiding problems with recombination between choice and performance genes. Despite it intuitive logic, there is virtually no empirical data to test this attractive idea.

POSTERS

Poster session 1 – Monday 27 May: 15:40-16:40

1.

Linking chemosensory multigene-family evolution with speciation in the pea aphid (*Acyrtosiphon pisum*).

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Within and between species, multigene families (MF) are known to be highly diverse in terms of both Copy Number Variation (CNV) and allelic diversity. This probably reflects the joint effects of specific new variant generating mechanisms and relatively widespread balancing – as well as divergent – selection experienced by MF. To date, most studies used either (i) multispecies phylogeny to focus on paralog and ortholog divergence at many genes across species; or (ii) population genetics and/or qPCR approaches to investigate diversity at few loci in single population studies. Thus, within and between population diversities have seldom been contrasted and their relative evolutionary dynamics remain poorly understood. The host-plant races of the pea aphid provide an excellent system to understand these dynamics in the context of speciation with gene flow. Notably, Chemosensory MF (CMF) – e.g. Olfactory Receptors (OR), Gustatory Receptors (GR), Odorant Binding Proteins (OBP) – are critically important for specific host plant recognition, i.e. putatively a main cause of reproductive isolation.

We sequenced 120 individuals from 8 races using a target enrichment protocol and Solexa sequencing – guaranteeing a median coverage of 150X. In doing so, we assessed CNV and nucleotidic diversity at about 3000 exons (from CMF, other MF and control genes) and 650 promoters of CMF genes.

Preliminary results show CNV is widespread, as it occurs in 65% of all exons (even surprisingly up to 57% for control exons). As with SNPs in single copy genes, most CNV in non CMF genes is shared among races. In contrast, for targets linked to chemosensory genes – notably GR exons and promoters – CNV tends to structure by race. Also, the rate of duplication appears higher for CMF: OR, OBP and promoters show significantly more CNV than other MF. Together, these results suggest that many CMF genes may evolve under positive selection and contribute to adaptation to host plants. In order to better understand the genetic basis of adaptation, work is ongoing to link patterns of CNV (i) to the history of gene flow between races; and (ii) to observed differences in gene expression within and between races across native and non-native host plants

2.

Linking macroevolution to ecology: how is the shape of phylogenetic trees affected by the ecological processes of diversification?

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Phylogenetic tree shape, which can be described as a combination of tree topology (tree balance) and diversification timing (branch lengths for time-calibrated trees), has been used as a testimony of the history of the diversification of clades since the 1970s. Since then, and now using larger and more accurate phylogenies, global patterns of macroevolution have been identified, showing variations between clades but global trends towards tree imbalance and towards early bursts followed by slowdowns in diversification. Various mechanisms have been proposed to explain the observed patterns of phylogenetic tree shape, either verbally or more recently using lineage-based models with time-, trait- or diversity-dependent evolutionary rates. However, the underlying ecological processes that may be responsible for these dependences have received little attention. Here, we report on how the shape of phylogenetic trees is affected by the ecological processes operating among individuals forming the evolving community. This requires using an individual-based model of diversification in which basic ecological interactions are incorporated. Our model also includes landscape dynamics – changes in the landscape geography over time whereby populations fragment and fuse recurrently. According to previous analyses, these landscape dynamics can indeed lead to evolutionary radiations, due to the alternation between allopatric phases of differentiation and sympatric phases of stabilization of coexisting species. Here, we analyze the shape of species trees generated by this model, and look at how features of the underlying ecological processes influence the predicted tree topology and timing of diversification.

3.

Widespread evidence for incipient ecological speciation

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Ecologically mediated selection is an important driver of speciation. The correlation between neutral genetic differentiation and environmental or phenotypic divergence among populations, to which we collectively refer to as isolation-by-ecology (IBE), is an indicator of ecological speciation. In a meta-analysis framework we determined the strength and commonality of IBE in nature. Using data from over 100 studies, we calculated a mean effect size of IBE with and without controlling for spatial autocorrelation among populations: effect sizes were 0.34 (CI_{95%} 0.24–0.42) and 0.26 (CI_{95%} 0.13–0.37), respectively. This indicates that an average of 5% of the neutral genetic differentiation among populations was explained purely by ecological contrast. Importantly, spatial autocorrelation influenced IBE correlations using environmental variables, but not phenotypes. Overall, our meta-analysis showed that ecologically induced genetic divergence is pervasive across timescales and taxa, and largely independent of the choice of molecular marker. We highlight the importance of these results in the context of adaptation and ecological speciation and highlight future research avenues and considerations.

4.

Are European oaks *Quercus petraea* and *Q. robur* a “worst-case scenario” for the study of speciation with gene flow?

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Until recently, very little was known about the nature, strength and genetic basis of reproductive isolation in sympatric *Quercus* species in Europe. Indeed, previous research spanning more than 20 years has focused essentially on the estimation of hybridization rates, and their significance in terms of new populations' establishment and introgression dynamics, despite acknowledged ecological divergence among species. This had led to the idea that reproductive barriers were “weak” in order to explain a fairly low average molecular divergence among species, and recent publications still put forward “high rates of interspecific gene flow” or “ongoing gene flow” between those two species. On the basis of recent results at both phenotypic and genomic levels, we argue that a low molecular divergence is compatible with strong reproductive barriers, that these barriers involve various mechanisms and are probably determined by a large number of genes, and that this suggests a very low realized gene flow in many established populations across both species' range.

5.

Evidence for strong isolating mechanisms despite pervasive hybridization in a European hybrid zone of *Primula veris* and *Primula vulgaris*

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Examining patterns of hybridization and introgression among lineages can assist with mapping the genetic architecture of reproductive isolating mechanisms. Natural hybrid zones provide an ideal setting to investigate the processes that maintain the genetic continuity of lineages in the presence of interspecific gene flow. The two common European *Primula* species *P. veris* (cowslip) and *P. vulgaris* (primrose) are known to hybridize in both laboratory and natural settings. Here we investigate the patterns of introgression between *P. veris* and *P. vulgaris* in populations that are part of a hybrid zone in the vicinity of Montreux, Switzerland, where morphologically intermediate forms are relatively abundant. Using nine new anonymous nuclear loci, we have genotyped hybrid and “pure” forms from several sympatric and allopatric populations of each parental species. Our results support the morphological identification of “pure” form *P. veris* and *P. vulgaris* and the classification of phenotypically intermediate individuals as hybrids. The relatively few loci employed make classification of the specific class of hybrid forms (i.e. F₂, backcross) problematic. Genomic and locus specific patterns of admixture reveal a somewhat contradictory portrait of interspecific gene flow, and this could be indicative of a relatively recent contact zone. We find high genetic

differentiation between *P. veris* and *P. vulgaris* which may be the product of very strong isolating mechanisms despite evidence for pervasive hybridization throughout their European range.

6.

Selection on male sex pheromone composition drives butterfly diversification

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Natural selection can facilitate diversification by inducing character displacement in mate choice traits that decreases the risk of non-adapted inter-lineage matings. Although reproductive character displacement has been demonstrated in two-taxa case studies, the frequency of this process in nature is still debated. The largely sympatric *Bicyclus* butterfly genus (Lepidoptera: Satyrinae) displays little morphological differentiation except for its striking diversity in androconia, the structures assumed to produce male sex pheromones. We showed recurrent character displacement in putative sex pheromone composition for 32 species, but not in other morphological mate choice traits. Moreover, sex pheromone composition appears to evolve faster than morphological traits. These results suggest that selection on divergence of male sex pheromones contributed to the diversification within *Bicyclus*. More generally, we stress that olfactory communication may play a more important role in species diversification than previously envisaged.

7.

The existence of species rests on a metastable equilibrium between inbreeding and outbreeding.

Etienne Joly

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I will present a new model whereby speciation results from natural selection. In other words, I will follow Wallace rather than Darwin, and will argue that reproductive barriers can be directly selected for.

According to this model, speciation will occur by budding, i.e. when small groups of individuals start breeding preferentially with one another, under the pressure of the ancestral stock, and thus necessarily in non-allopatric conditions.

For such budding groups, two of the most direct disadvantages of breeding back with the ancestral stock would be the loss of advantageous, but recessive phenotypes, and the recombination load, i.e. the disruption of advantageous gene combinations.

Contrarily to a very commonly held view, inbreeding does not have only disadvantages, especially in the long run, and, according the model proposed, extensive outbreeding could contribute to species extinction due to excessive accumulation of recessive mutations, whilst strong tendencies for inbreeding would lay the ground for rampant speciation, and consequently to short-lived species because of a high turnover.

8.

Trophic morphology differences in three sympatric Arctic charr morphs in Lake Skogsfjordvatn

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Arctic charr (*Salvelinus alpinus*) is a phenotypically plastic species that can occur as different morphs in sympatry in the same lake. The different morphs are typically adapted to utilize different habitats and dietary niches.

We have used geometric morphometric methods to examine three different morphs of Arctic charr in Lake Skogsfjordvatn, Northern Norway. The lake is about 13 km² and has large areas with depths between 25 to 100 meters. The three morphs of Arctic charr are distinctly separated ecologically (habitat preferences and diet) and genetically. There is one littoral spawning omnivore morph (LO), one profundal spawning bentivore morph (PB) and one profundal spawning piscivorous morph (PP).

Using morphological tools we revealed significant differences in body shape and head shape between the three morphs. A PCA-analysis show that the two deep-water PB and PP morphs have a bigger head and a relatively shorter body length than the upper water LO morph. The PB morphs were found to have a blunt head shape with a sub-terminal mouth position, a small maxilla and large eyes. The PP morphs have a pointed head shape with a

terminal mouth position, a long maxilla and intermediate eye size. The LO morph had the smallest eyes. The dorsal and the ventral fins are also situated further back on the body of the PB and PP morphs compared with the LO morph.

We found that these profound head and body shape differences was evident through the entire ontogeny of the morphs from the smallest fish (< 15 cm), the medium fish (15-25 cm) to the largest fish (>25 cm) by using CVA and Mahalanobis distances. Regarding body shape, the most differing size group of the morphs was between the large LO and the small PP, while the most similar body shape was found between subsequent size groups within a morph. Regarding head shape the largest differences was found between the small PB and the biggest of the PP-morph. The most similar head shape among the size groups were found between subsequent size groups within a morph. The ontogenetic differences in head and body shape between morphs, increased by increasing size but was also very distinct between similar size groups. The results evidently show that the body and head shape of the morphs is quite differentiated already when they are juveniles, suggesting a genetic component involved. The head shape was relatively similar between the three morphs within the smallest size group.

9.

A transcriptomic perspective on past hybridisation in the fire-bellied toads (*Bombina*)

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The two anciently diverged European fire-bellied toads *Bombina bombina* and *B. variegata* occupy adjoining geographic ranges and are adapted to different breeding habitat. Despite their profound ecological divergence, which is reflected in numerous phenotypic differences, these taxa still produce abundant fertile hybrids in narrow contact zones. As a consequence of range expansions and contractions over the last 5-6 million years, such conduits for introgression are likely to have existed repeatedly. We aim to detect the genetic signature of past introgression from *de novo* assembled transcriptomes of both taxa based on 454 and Illumina sequence reads. Additional Illumina assemblies were generated from the nearest relative, *B. orientalis*, and from a separate Balkan lineage, *B.v. scabra*. From these four datasets, orthologous gene sets were extracted. We present data on the structure of these transcriptomes and compare the variance in coalescence times between hybridising and non-hybridising taxa.

10.

Drang nach Westen: Is the fire-bellied toad a ring species?

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Dispersal from multiple glacial refugia resulted in a mosaic of parapatric clades in *Bombina*. In a lowland bound *B.bombina* AMOVA identified 3 geographic groups: the Northern, Southern and Anatolian one originating from presumed peri-Black Sea refugia. The N and S lineages experienced spectacular post glacial expansion to the west circumnavigating the Carpathians and Sudety Mts along remodeled river systems. The two branches met in Central Bohemia, in a secondary contact, as revealed by lineage specific codon deletions in their divergent mtDNAs. This contact offers a unique opportunity to study predictions of divergence with distance model along environmental gradients in a species involved also in hybridization with *B.variegata*.

11.

Constraints on ecological speciation in *Murella muralis*: environmental changes in the course of time

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The debate about speciation and diversification currently focuses on the relative importance of factors driving population differentiation. A point that deserves empirical exploration is how and to what extent historical factors

affect speciation in interactions with selection and in the presence of environmental changes. To investigate these questions, we quantified variation on a fine geographical scale analysing morphological (shell) and genetic datasets coupled with environmental data in the land snail *Murella muralis* (Müller 1774), endemic to the Mediterranean island of Sicily. The species exhibits an impressive morphological diversity with shell shapes that vary from globular to keeled-flat, even over relatively restricted geographical areas. This spectacular variation in shell shapes has been documented in many other groups of land snails, however its evolutionary causes are not yet understood. Analysis of a fragment of the mitochondrial DNA Cytochrome Oxidase I gene (COI) and eight nuclear microsatellite loci showed that genetic variation is highly structured at a very fine spatial scale by local palaeogeographical events and historical population dynamics. We showed that globular and keeled-flat shells were generated by divergent selective forces. In particular, keeled-flat shells are an adaptation to dry open habitats along the coast during the Plio-Pleistocene. Our data suggested that shell differentiation has been driven by divergent adaptation to a patchy (in time and space) environment in which woods alternated with open arid habitats. This patchiness drove differentiation, while palaeochanges in landscape caused populations to repeatedly come into contact and separate. We suggest that time and environmental changes in the course of time acted as constraints on the ecological speciation. Follow-up researches on the genomic of speciation and constraints on it in this study system are in progress and are briefly presented.

12.

Genetics and genomics of speciation in the brown seaweed *Ectocarpus* complex

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Understanding the genetic basis of speciation is a long standing goal in evolutionary biology, but many questions remain unanswered or debated such as the link between adaptation to divergent environments and the evolution of reproductive isolation (Arnold 1997; Rieseberg 1997; Orr et al. 2004, Mallet 2005). This area is very challenging especially for the marine realm, since important gaps exist in our understanding of speciation compared to terrestrial organisms (Miglietta et al., 2011). The filamentous alga *Ectocarpus* is a model organism for the genetics and genomics of brown algae (Peters et al, 2004, Cock et al., 2010). It has a cosmopolitan distribution and systematics and taxonomy studies, based on a worldwide collection, demonstrated that it is a complex of closely related species separated by various degrees of intersterility (reviewed in Stache-Crain et al. 1997). Natural hybrids have been documented between *E. siliculosus* and *E. crouaniorum* both in France and Chile (Peters et al. 2010 a, b) which makes these two species suitable case to study adaptive divergence and reproductive isolation. In this poster, we present the principal objectives of our research project. We aim to exploit the genomic and genetic tools that are being developed for this alga to study the genetic basis of speciation in this species complex. First, we propose to combine detailed analyses of gene flow across natural hybrid zones (using a set of microsatellite loci) with the study of reproductive isolation in the laboratory. Second, next generation sequencing (NGS) methods will be used to identify chromosomal regions contributing to reproductive isolation (comparison among species) and to adaptive divergence (comparison among strains along environmental gradients).

Poster session 2 - Tuesday 28 May 17:00-18:00

13.

Genetic structure of *Pterodroma leucoptera* complex: A multilocus inference

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Studies of *Pterodroma leucoptera* complex seabird were conducted by different authors in order to assign species boundaries among its morphosubspecies. In this study, “fresh” and “museum” samples were used in order to recover sequences from mitochondrial genes (Co1 and Cytb) and nuclear introns (PAX, CSDE, Bfib, TPM, Lam, RP40, and IRF2). The genetic structure of ten populations of *P. leucoptera* complex were studied in order to (i) assess degree of population differentiation and ii) to infer phylogenetic relationships among them. A range of phylogenetic (Maximum Likelihood, Bayesian multilocus) and phylogeographic (molecular diversity Fu's Fu, Tajimas'D) analyses were conducted to uncover molecular boundaries inside this group. Two main clades were differentiated: *P. leucoptera* and *P. brevipes*. Within *P. brevipes* group two sub clades were found: one corresponding to Vanua Lava *P. brevipes magnificens* and the other grouping Eromango and Society populations of *P. brevipes* morphosubspecies. This study reinforces the qualification of species status of the two main clades found inside *P.*

leucoptera complex as well as the presence of substructuring within *P. brevipes* morph-species.

14.

When iguanas meet: Hybridization, introgression and cytonuclear discordance in contact zones of the Mexican black iguana

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Ctenosaura pectinata, an iguana endemic to Mexico, has been revealed to be composed of several highly divergent mtDNA lineages with some of them forming contact zones, and one of them overlapping with *C. hemilopha*. We assessed and compared the dynamics of gene flow at these inter- and intra-specific contact zones using mtDNA and microsatellite data. The mtDNA contact zone between *C. hemilopha* and *C. pectinata*, spans 12 km with no clear association with environmental change. Hybridization between these species is confirmed by the existence of F1 and backcross individuals. Weak signals of prezygotic isolation were detected that might account for the observed pattern of asymmetrical mtDNA introgression of *C. hemilopha* into *C. pectinata*. To study intraspecific contact zones within *C. pectinata*, a transect across four overlapping mtDNA clades was sampled. Microsatellite data revealed only two nuclear gene pools, indicating that nuclear and mtDNA data do not follow concordant patterns within this region. The discordance can be explained in part by introgressive hybridization together with ongoing nuclear gene flow via male-biased dispersal. Overall, patterns of gene flow reflect the time since isolation of the gene pools involved, with narrow and steep mtDNA and nuclear clines in the interspecific hybrid zone, in contrast to a wider area of overlap between intraspecific mtDNA lineages and extensive nuclear introgression. With the recent development of Next generation technologies, *Ctenosaura* constitutes an excellent system to address questions on genomics of speciation at different scales of divergence.

15.

ABGD, Automatic Barcode Gap Discovery for primary species delimitation

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Within uncharacterized groups, DNA barcodes, short DNA sequences that are present in a wide range of species, can be used to assign organisms into species. We propose an automatic procedure that sorts the sequences into hypothetical species based on the barcode gap, which can be observed whenever the divergence among organisms belonging to the same species is smaller than divergence among organisms from different species. We use a range of prior intraspecific divergence to infer from the data a model-based one-sided confidence limit for intraspecific divergence. The method, called Automatic Barcode Gap Discovery (ABGD), then detects the barcode gap as the first significant gap beyond this limit and uses it to partition the data. Inference of the limit and gap detection are then recursively applied to previously obtained groups to get finer partitions until there is no further partitioning. Using six published data sets of metazoans, we show that ABGD is computationally efficient and performs well for standard prior maximum intraspecific divergences (a few per cent of divergence for the five data sets), except for one data set where less than three sequences per species were sampled. We further explore the theoretical limitations of ABGD through simulation of explicit speciation and population genetics scenarios. Our results emphasize in particular the sensitivity of the method to the presence of recent speciation events, via (unrealistically) high rates of speciation or large numbers of species. In conclusion, ABGD is fast, simple method to split a sequence alignment data set into candidate species that should be complemented with other evidence in an integrative taxonomic approach

16.

Phenotypic plasticity can promote sympatric speciation

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Allopatric speciation has traditionally been viewed as the most likely mechanism for the development of reproductive isolation, hence speciation. Recent studies advocate that ecological speciation (speciation by ecologically based divergent selection) in sympatry is also a plausible mechanism contributing to speciation. Phenotypic plasticity has been suggested as a factor that can facilitate ecological speciation, but the efficacy of plasticity in promoting speciation is debated in literature; it can theoretically either increase or decrease the likelihood of speciation. One possibility is that plasticity can increase phenotypic variation in the population, which in turn may facilitate assortative mating. The objective of this paper is to examine the conditions under which phenotypic plasticity could promote or hinder sympatric speciation and the underlying mechanisms. We developed a stochastic individual based simulation model of a sexually reproducing predator population and asexual prey populations. In the model, the ecological (foraging) trait and phenotypic plasticity of the predator can evolve in response to food availability. Individuals can exploit two prey types, and attack rate and handling time depend on predator phenotypes. Mating can occur in a common or separate mating ground.

We show that sympatric speciation occurs more quickly when phenotypic plasticity is allowed to evolve. This happens under relatively strong assortative mating and moderate habitat sensitivity. Elevated phenotypic variation increases the efficacy of assortative mating and consistency in habitat choice over time. When the population has enough time to evolve sufficient plasticity before it reaches the evolutionary branching point (where selection switches from directional to disruptive), the population is more likely to stay monomorphic. These are, therefore, two alternative evolutionarily stable states in this system. We conclude that the presence of relatively low plasticity can speed up sympatric speciation when assortative mating is sufficiently high. Phenotypic plasticity can also hinder sympatric speciation if individuals possess high plasticity when the population experiences disruptive selection.

17.

Has a third taxon of *Chorthippus parallelus* arisen in its Pyrenean hybrid zone?

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Speciation by hybridization, sympatric speciation and reinforcement remain controversial issues in evolutionary biology, but the possibility that they can occur is becoming more widely accepted as evidence of speciation in the face of gene flow accumulates.

The Pyrenean hybrid zone between *Chorthippus parallelus parallelus* and the Iberian endemism *Chorthippus parallelus erythropus* (Orthoptera) was originally described as a “secondary contact zone”, maintained by the balance between dispersion and the presumed reduced fitness of the natural hybrids. This is supported by the close adherence to Haldane’s rule (whereby heterogametic males are sterile) of F1 laboratory hybrids of pure individuals of the two subspecies, their mating behaviour and the homogamy detected in studies of females’ sperm preference.

However, recent cytogenetic and morphometric findings suggest a need to revise our view, also supported by further data concerning (1) the distribution of certain microsatellites in the hybrid zone, (2) the different patterns of infection by the bacterial endosymbiont *Wolbachia* in hybrid populations, and (3) the distinct influence on spermatid formation induced by this bacterium in natural hybrids.

An estimated 10,000 generations of hybridization have occurred naturally in the Pyrenees, possibly resulting in not only the progressive dilution of the genomic incompatibilities between the two subspecies, but also the appearance of “rare alleles”, which are characteristic of hybridization processes (to which the cytogenetic evidence attests), and the coevolution of the compatible components of the two original genomes, to yield a third taxon that is distinct from both pure subspecies. Our current evidence for this proposal is presented and discussed.

18.

Genital divergence in sympatric sister snails

[J. Hollander](#), C. M. Smadja, R. K. Butlin, & D. G. Reid

Speciation is the evolutionary process in which new species originate by the splitting of existing lineages. While it is recognised that reinforcement is one of the possible mechanisms of speciation, a major challenge remains to test the importance of this process in nature. However, few large-scale comparative analyses have addressed this prediction, especially for genital form. Here we present an exceptionally complete and robust phylogeny to underpin detailed

analysis of the form of male genitalia in the marine gastropod family Littorinidae (periwinkles). Our study of sister-species pairs has found a strong pattern that could be a signature of the controversial process of reinforcement: the form of the elaborated male genitalia is more divergent between species pairs with overlapping geographical distributions than between allopatric pairs.

19.

Sexual selection against natural hybrids: a barrier to gene flow in a house mouse hybrid zone?

Yasmin LATOUR, Marco PERRIAT-SANGUINET, Pierre CAMINADE, Pierre BOURSOT, Carole M. SMADJA, Guila GANEM

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Sexual selection could contribute to reproductive isolation when hybrid mate recognition signals are discriminated against. We tested this hypothesis in a unimodal hybrid zone between two subspecies of the house mouse (*Mus musculus musculus* and *M. m. domesticus*) showing assortative mating. We performed habituation-discrimination tests indicating that the hybrid signals might be transgressive. Further, we presented mice from the border of the hybrid zone with a choice between opposite sex urine, from the same subspecies versus from hybrid sampled in different locations across the zone. While no preference was evidenced in a sample of *M. m. domesticus* mice, *M. m. musculus* discriminated against hybrid signals in favor of their own subspecies and the pattern did not seem to vary significantly across the hybrid zone. However, *M. m. musculus* mice sampled in allopatric locations close to the hybrid zone did not show any assortative preference. This pattern of character displacement suggests that sexual selection against hybrids is a local adaptation that could have evolved in parallel or as a by-product of reinforcement. We conclude that asymmetric sexual selection against hybrids could impact both isolation between the two subspecies and the hybrid zone dynamics.

20.

How do co-occurring sister species deal with spatial, reproductive and dietary overlap?

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The evolution of species could be driven by selection (natural or sexual) via, for example, divergence in habitat selection and mate recognition systems. When sister species share the same environmental niche in allopatry their co-occurrence in sympatry results in at least one species showing character displacement. We test this hypothesis in the African striped mouse, endemic to southern Africa. The genus *Rhabdomys* is composed of 6 mitochondrial clades that radiated around 4.3 Ma years ago. The species status of these clades is unresolved. However, two taxa, *R. bechuanae* and *R. dilectus dilectus*, have a consistently high mitochondrial and nuclear divergence, suggesting that they are distinct species. Interestingly, these species are parapatric occurring in areas characterized by distinct precipitation, temperature and vegetation gradients, although several contact zones were recently found where the two sister species occur in environments with similar characteristics. Such ecological and geographical settings provide a natural context in which to assess the plasticity of the environmental niches of the two species and the impact of their potential interaction in sympatry on this niche. We first did a spatial analysis to investigate habitat overlap at a micro-geographical scale, using radio-tracking data and analysis of home range size and overlaps between and within each species in sympatry and allopatry, and under different environmental conditions. In order to test sexual discrimination between the two species, we performed sexual preference tests and dyadic encounters of individuals. We also evaluated resource holding potential using intra-sex behavioral dyadic encounters between and within the species presenting the two interacting mice with their favorite food after slight deprivation. Finally, we assessed diet variation across habitat types and species using δC^{13} and δN^{15} isotopic analyses of the fur of free-living striped-mice. These results are currently being analyzed and already suggest that potential for competition may exist.

21.

Simulating neutral genetic divergence during ecological speciation

Michael Kopp and Laurine Costa

Aix-Marseille University

Diverging populations or incipient species are expected to accumulate genetic differences. At neutral loci, the amount of divergence depends on their distance to selected loci and the overall amount of gene flow, which in turn is determined by migration, selection, and potentially assortative mating. We will present results from individual-based

simulations that have been designed to predict Fst-values at microsatellite markers in simple models of ecological speciation with gene flow. These results will be compared to previously published analytical approximations. A long-term goal is to apply this model to the analysis of empirical data from diverging populations in nature.

22.

Genetics and epigenetics contributions to the host plant adaptation of two Lepidoptera pests

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Phytophagous insects provide key examples of diversification by ecological speciation. For ecological speciation to occur, three components are necessary: 1) a source of divergent selection, 2) a form of reproductive isolation either pre- or post-zygotic, and 3) a mechanism linking selection to reproductive isolation. This latter mechanism takes place either at the phenotypic level, as e.g. a shift in the insect mating period driven by the host plant phenology, or at the genetic level, as e.g. same genes underlie recognition of the plant and of the sexual partner. We will focus on this genetic scale of the insect/plant relationship in two complexes of moth species.

We will study on the one hand, the European corn borer (ECB), *Ostrinia nubilalis* and its sister and sibling species, the Adzuki bean borer (ABB), *Ostrinia scapularis* and on the other hand, two host races of *Spodoptera frugiperda* (called the "rice strain" and the "corn strain"). These borers species and fall armyworm races share some common traits: they are sympatric while genetically differentiated and specialized on different hosts (maize for ECB and mugwort and hop for ABB; rice and corn for the so-called 'rice strain' and 'corn' strain of *Spodoptera*). This specialization concerns two main characters: the larval performance and the choice for oviposition site. All these features make them appropriate candidates for ecological speciation by host plant specialization.

We developed a transcriptomic approach to detect genes or gene families involved in the specialization (and speciation) of these two moth complexes. We first proceed to controlled infestation in semi-natural conditions for each *Spodoptera* strain or each *Ostrinia* species on their usual host and on an alternate host. Life history traits will be measured throughout a life cycle and RNA pools of larval tissues for the different modalities (moth/host) will be sequenced by NGS technologies. Genes, families of genes and genetic pathways will be compared between modalities in order to characterize the genetic response to the host-plant specialization. In particular, we will focus on the regulatory regions of those differentially expressed genes and identify the genomic and epigenomic variations between species or strains (SNPs, indels, breakpoints, histone modifications, etc...) that may effect the gene expression at these loci. In conjunction with population genetics study, we hope to identify this way the common or divergent molecular mechanisms at play in the speciation mechanisms of our two incipient species models.

23.

Wolbachia infection in *Podisma pedestris*

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We investigate the *Wolbachia* infection in *Podisma pedestris*. This grasshopper is subdivided into two chromosomal races with different sex determining systems. A region of hybrid inviability occurs between the two races in the Alps, which has previously been interpreted as the result of recombination of many coadapted genes. Here we pursue the alternative (or additional) possibility that the inviability is due to effects of *Wolbachia* infection. Both current and ancient infection can be characterised because there has been extensive lateral gene transfer from live *Wolbachia* into the *Podisma* genome. Amplicon sequencing of nuclear inserts from selected populations across the zone allows us to infer the date and geographic location of the insertion events.

24.

Adaptive radiations in alpha and beta niche dimensions - The potential role of extinctions for patterns of niche conservatism

Jörgen Ripa

Adaptive radiations are interesting and important, and probably more than just a sequence of speciations. Recently, there has been an increased interest in the more exact sequence of events, how a clade develops from its first ancestral population to a group of closely related species, adapted to different niches in the landscape in which they

occur. Some traits seem to be conserved through evolutionary history, whereas some are more labile, with little phylogenetic signal. The concepts of alpha and beta niches, corresponding to within- and between-habitat selection, has made it possible to hypothesize that one set of traits diverge sooner than others. However, data points in different directions. I here present a first attempt to disentangle the mechanisms underlying the sequence of trait divergence. A first, tentative, prediction is that traits with the strongest trade-off will diverge first. However, model simulations show that this pattern is rather contingent on initial conditions and the amount of available genetic variation in different traits. Somewhat surprisingly, much more robust conclusions can be drawn if random extinctions are allowed to occur during the adaptive radiation. This has direct consequences for how to interpret patterns of niche conservatism.

Poster session 3 – Wednesday 29 May 15:40-16:40

25.

Repeated niche occupancy through species sorting and parallel radiation are not always so different: insights from a Galapagos beetle radiation.

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When environmental gradients are repeated on islands, habitat occupancy may result from either species sorting, wherein each ecotypic species colonizes the different islands, or through independent parallel adaptive radiation. However, it remains questionable whether island monophyly, often interpreted as evidence for *in situ* adaptive radiation, could be the result of a species sorting mechanism followed by extensive levels of introgression within islands and may strongly impact our understanding on the relative roles of determinism and contingency in evolution. On the Galapagos, three distinct species of *Calosoma* caterpillar hunters sharing phenotypic traits are found exclusively on higher elevations of different islands, while lowlands are occupied by a fourth species. Although highland and lowland species are indeed remarkably closely related on most islands suggesting a recent parallel radiation, conflicting results from mitochondrial haplotypes demonstrate that highland species of two islands were initially monophyletic, but followed by extensive amounts of horizontal gene exchange with the lowland species. These findings not only relax the use of neutral gene genealogies to prove parallel evolution, but moreover indicate that interspecific gene exchange within as well as among islands makes the distinction between niche occupancy through adaptive radiation and species sorting less clear-cut as previously assumed.

26.

Does intrasex competition interfere with reproductive character displacement in the house mouse?

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Reproductive Character Displacement (RCD) often results from selection for better discrimination of compatible conspecific mates. However it has been pointed out (Grether et al, 2009) that same-sex interactions, such as interference competition, can also lead to the evolution of species recognition systems. Displacement of species recognition signals and associated mate preferences was evidenced along a secondary contact zone between two subspecies of the house mouse. This pattern was explained by selection against maladaptive hybridization, and differences in competitive abilities were suggested as a possible cause of higher choosiness in the less competitive subspecies which endures more selective pressure on mate choice. However, those differences could also impact species recognition divergence directly through ecological character displacement. We present the results of a study designed to test the latter hypothesis. If competitive interference occurs in the contact zone one may expect avoidance of agonistic interactions or increased aggressiveness of the subordinate subspecies. Our ongoing experiments compare agonistic characters (aggressiveness, dominance, competitor avoidance) in allopatric and sympatric populations of the two subspecies, during intraspecific and interspecific male dyadic encounters. If displacement of behavioural traits involved in dominance is evidenced in the contact zone the role of competition in shaping the species mate recognition system would have to be considered. Further, given that female mice perceive male dominance as a quality trait, dominance may also influence mate choice. For the less competitive subspecies, potential for a conflict of information between quality and compatibility signals exists and could have also shaped divergence of mate recognition in the contact zone. We test this hypothesis assessing female preference for dominant males in contact zone and allopatric populations of the subordinate subspecies and investigate whether and how this apparent dilemma is solved in the Danish contact zone

27.

Adaptive divergence among incipient species: a parasite perspective

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The radiation of cichlid fishes in the East African Great Lakes represents one of the most powerful study systems in speciation research. Although the phylogeny of the species flocks have been described in detail, the mechanisms of incipient speciation remain largely unexplored. In Lake Tanganyika, allopatric colour morphs of the philopatric genus *Tropheus* have been reported to be partially reproductively isolated. As reproductive isolation among incipient species has been proposed to be induced by adaptation to contrasting environments, we wanted to know, whether and to which extent *Tropheus* colour morphs are locally adapted. From six populations along the Zambian shoreline of Lake Tanganyika, we screened a representative sample of *Tropheus* for endo- and exoparasites. We found significant spatial structure in the composition of these parasite communities across host populations. Furthermore, we found local adaptation at the level of immunogenes of the major histocompatibility complex (MHC). In contrast, we failed to detect such a pattern in *Simochromis diagramma*, a closely related, sympatric cichlid with a higher dispersal capacity. It confirms that the degree of philopatry is an important driver of both environment (parasite community) and local adaptation. The proposed pleiotropic role of the MHC in parasite defense and assortative mating makes us speculate, whether local parasite communities could drive speciation in *Tropheus* as by-product of reproductive isolation.

28.

Species delimitation and phylogeny in the pest genus *Nasutitermes* (Termitidae: Nasutitermitinae) in French Guiana

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Species delimitation and identification for taxa whose morphologic characters are confused can be arduous and are likely to hamper global biodiversity assessments and pest species management. Exploratory methods of species delimitation, using DNA sequence itself as the primary information source to establish group membership and estimate putative species boundaries, represent useful preliminary treatment for traditional taxonomy. Termites of the genus *Nasutitermes* are dominant in Neotropical primary forests but also represent major agricultural and structural pests. Despite their prevalence, pivotal ecological role and economical impact, the taxonomy of *Nasutitermes* species mainly depends on often unreliable characters of soldier external morphology. Even widespread species such as *N. corniger* are often misidentified and highly specialized expertise is required to survey species. Here, we generated robust species hypotheses for 79 *Nasutitermes* colonies sampled throughout French Guiana without a *priori* knowledge of species affiliation. Barcode analysis of *cytochrome oxidase II* gene was coupled with species delimitation tools using the automatic barcode gap discovery method (ABGD) and a generalized mixed Yule coalescent model (GMYC) to propose primary species hypotheses (PSHs). PSHs were reevaluated using two more loci (*mitochondrial 16S rDNA* and *nuclear internal transcribed spacer 2-ITS2*) and morphotypes leading to 16 retained secondary species hypotheses. Seven of them, represented by 44 colonies, were morphologically affiliated to species reported as pests in the Neotropics. Multigenic phylogenies were also reconstructed to identify ancestral ecological niches and major-pest lineages.

29.

Characterization of secondary contact zones and hybridization patterns in the tellinid bivalve *Macoma balthica*

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Little is known on the genes and gene interactions involved in inter-population genetic divergence in the context of

secondary contact. The marine bivalve *Macoma balthica* has a complex biogeographic history in the NE Atlantic, punctuated by multiple events of trans-arctic invasions and the establishment of secondary contact zones. Here we present our efforts to characterize the structure of these contact zones and the nature of the barriers to gene flow (endogenous / exogenous). One mitochondrial gene, seven microsatellites, and 384 SNPs were used to characterize the genetic structure in Europe, particularly across two genetic breaks (Bay of Biscay, and entrance of the Baltic Sea). Genetic markers revealed a hierarchical genetic structure in Europe, with barriers to gene flow mainly focused around Brittany, and the entrance of the Baltic Sea. In addition, we used transcriptome-wide DNA sequence data to explore patterns of divergence and selection across divergent *M. balthica* populations, with emphasis on genes involved in mitochondrial processes. F_{ST} scans revealed multiple nuclear genes coding for mitochondrial-targeting proteins as highly differentiated among populations. Analysis of dN/dS ratios revealed that 5% and 45% of the 752 tested genes were under positive and negative selection (respectively), with genes involved in mitochondrial energy production detected in both categories. Over 87% of genes coding for mitochondrial-targeting proteins were under negative selection. Gene ontology terms associated with the mitochondrial machinery and multiple binding activities (including ATP binding) are enriched across populations. There is therefore accumulating evidence suggesting that mitochondrial-targeting proteins and their interactions play a role in maintaining population divergence in *M. balthica*. Eight mitochondrial genomes are therefore being sequenced to look for patterns consistent with mito-nuclear incompatibilities.

30.

Feeding behavior of *Acyrtosiphon pisum* races on different legume species

Grit Kunert

Alexander Schwarzkopf, Jonathan Gershenzon, Grit Kunert.

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The pea aphid (*Acyrtosiphon pisum* Harris) consists of over ten genetically distinct host races, each native to a single species of the Leguminosae. Yet all thrive on broad bean *Vicia faba*. This ecological specialization can be considered as one step towards sympatric speciation. Host fidelity leading to assortative mating seems to be an important mechanism that reduces gene flow between host races.

We localized plant factors which influence host fidelity and hence host race formation in *A. pisum*. In a broad comparative study on several *A. pisum* clones collected from different legume host plants in France and the United Kingdom, we characterized the performance of the *A. pisum* clones on different legume species, and showed different degrees of specialization to potential host plant species. Afterwards, we monitored the feeding behavior of six aphid clones from three races on four plant species by the Electrical Penetration Graph (EPG) technique. The differences we found in feeding behavior can explain the different degrees of host plant specialization. We also identified the most important plant factors influencing aphid feeding behavior to be located in the epidermis and sieve elements.

31.

Investigating ecological hybrid unfitnes between host-specialized biotypes of the pea aphid

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Adaptation to distinct niches can cause reproductive isolation and genetic divergence between populations. Even though ecological speciation may be common, it is difficult to distinguish the role of ecology from possible non-ecological factors contributing to reproductive isolation. These “intrinsic” barriers may prevent gene flow between related species at any particular stage of their reproductive cycle. This is why ecological speciation is studied at early stages of the process, below the “species level”, when it cannot be asserted that speciation will eventually be complete.

We aim at tackling this difficulty by using biotypes of the pea aphid complex (*Acyrtosiphon pisum*), which feed on distinct species of legumes (Fabaceae). Each biotype presents very low fecundity when fed on host plants of other biotypes. However, broad bean (*Vicia faba*) is favorable to all biotypes in lab conditions. The various levels of divergence of pea aphid biotypes (comprising host races and species), and the existence of a universal host offer a rare opportunity to distinguish the influence of host-plant adaptation from that of intrinsic defects on hybrid fitness, and how their respective contribution varies in the course of speciation. Preliminary results from more than 150 F1 crosses between 5 pea aphid biotypes are presented and discussed.

32.

Does “Coyne’s Rule” apply to amphibians? Introgression of sex-linked and autosomal markers across a tree frog hybrid zone

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The large role of sex chromosomes in reproductive barriers between newly evolved species is one of the best-supported ideas in speciation research, and has been termed “Coyne’s Rule”. Most of this support, however, comes from studies of organisms with well-differentiated sex chromosomes such as fruit flies, mammals, and birds. In contrast to these groups, many fish and nearly all amphibians have undifferentiated sex chromosomes, which contain a small sex-determining region and very large pseudo-autosomal regions. To date, little evidence is available to test how this dramatic difference in sex chromosome structure affects the genetic architecture of reproductive barriers. We used genotyping-by-sequencing to estimate population allele frequencies for 2506 SNPs at 30 localities across the hybrid zone between *Hyla arborea* and *Hyla orientalis*, and estimated cline width and position in order to test for differential introgression. Our results show no significant difference between introgression of sex-linked and autosomal markers, suggesting that the large effect of sex chromosomes on speciation may be limited to species with old and highly divergent sex chromosomes.

33.

Sympatric divergence mediated by standing genetic variation and a potential automatic magic trait in the salt marsh beetle *Pogonus chalceus*

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It is assumed that adaptation to different ecological conditions in sympatry is only plausible under certain, more stringent, conditions that reduce the blending effect of gene flow.

Western European populations of the salt marsh beetle *Pogonus chalceus* are characterized by large interpopulation variation at various geographical ranges in at least two traits related to dispersal power, i.e. wing size and different allozymes of the mitochondrial NADP⁺-dependent isocitrate dehydrogenase (*mtldh*) gene. Short-winged populations are mostly found in stable tidal marshes, long-winged populations are found in temporary inland marshes. Moreover, in the Guérande region in France, strongly differentiated population (wing size and *mtldh*) are found in a sympatric mosaic in which the stable and temporary habitats are found in hundreds of replicates and only 10-20m apart.

First, we demonstrate that the divergence in wing size is highly genetically determined and that divergence between these populations may involve multiple unlinked loci.

Next, using sequence variation, we show that the repeated and sympatric divergence is based, at least for the *mtldh* region, on introgression of standing genetic variation. Furthermore, the old divergence between the differently selected *mtldh* alleles suggests a long term conservation of this genetic variation, which has been inferred to recently spread throughout the Atlantic area into stable populations.

Finally, using inundation experiments, we demonstrate that allopatric long-winged populations show a higher reluctance to submersion than short winged populations. This reluctance may be involved in habitat separation, which on its turn could promote the preservation of this sympatric system.

34.

The paths of parallel evolution in the flat periwinkles: preliminary results

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It is often assumed that the genetic mechanisms underlying repeated divergent evolution are entirely independent. However, many studies favouring this view relied exclusively on the analysis of neutral markers, which do not allow to rule out alternative hypotheses such as: selection on shared standing genetic variation, spread of advantageous

alleles over large geographic scales, and spread of advantageous alleles from closely related sympatric/parapatric species. Characterized by abrupt changes in the environmental conditions over a narrow spatial scale, the marine intertidal is an ideal system to provide valuable insights into parallel ecotype evolution and ecological speciation. Marine intertidal snails of the genus *Littorina*, exposed to those abrupt changes, have been shown to be well suited for studying the evolution of local adaptation. This is particularly the case of the flat periwinkle *Littorina fabalis*, a common species distributed along the NE Atlantic coast, which presents different locally adapted ecotypes throughout Northern Europe and the Iberian Peninsula, providing an interesting system to address the mechanisms involved in parallel ecotype evolution. This is the focus of an interdisciplinary ongoing project that aims to shed light on the mechanisms of ecotype evolution and to understand if parallel divergent evolution on this species evolves through the same genetic paths. We will describe the main axes of this research project and present some preliminary results.

35.

Speciation in a large tropical amphibian radiation

David R. Vieites, Sandra Nieto-Román, Marcos Peso, Nina Bernard.

The native amphibian fauna of Madagascar is constituted by five endemic evolutionary lineages of frogs with nearly 100% species-level endemism. Those include the endemic family Mantellidae that shows a wide species diversity within the island. Two undescribed mantellid species were also known from the oceanic Comoros Islands, which were colonized twice by oceanic dispersal ca. 6.5 mya. The recent discovery of a third species of mantellid frog in Comoros, sister and syntopic to one of the known Comoroan species, suggest that they have evolved in sympatry within the island. We here present phylogenetic, morphological, ecological and bioacoustic data of these species and discuss them within speciation scenarios that have been proposed for Mantellid frogs.