Drosophila evolution:

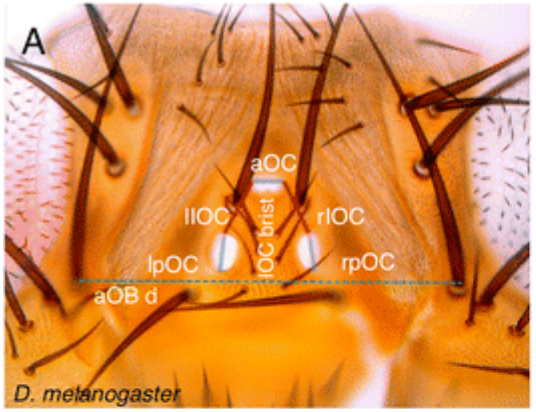
Toward a study of gene regulatory constraints to morphological evolution of the *Drosophila* ocellar region

Aguilar-Hidalgo, D., Becerra-Alonso, D., García-Morales, D., and Casares, F. (2016).

Dev. Genes Evol. *226*, 221–233.

The morphology and function of organs depend on coordinated changes in gene expression during development. These changes are controlled by transcription factors, signaling pathways, and their regulatory interactions, which are represented by *gene regulatory networks* (GRNs). Therefore, the structure of an organ GRN restricts the morphological and functional *variations* that the organ can experience—its potential morphospace.

Therefore, two important questions arise when studying any GRN: **what is the predicted available morphospace** and **what are the regulatory linkages that contribute the most to control morphological variation within this space.** Here, we explore these questions by analyzing a small “three-node” GRN model that captures the Hedgehog-driven regulatory interactions controlling a simple visual structure: the ocellar region of *Drosophila*. Analysis of the model predicts that random variation of model parameters results in a specific non-random distribution of morphological variants. Study of a limited sample of drosophilids and other dipterans finds a correspondence between the predicted phenotypic range and that found in nature. As an alternative to simulations, we apply Bayesian networks methods in order to identify the set of parameters with the largest contribution to morphological variation. Our results predict the potential morphological space of the ocellar complex and identify likely candidate processes to be responsible for ocellar morphological evolution using Bayesian networks. We further discuss the assumptions that the approach we have taken entails and their validity.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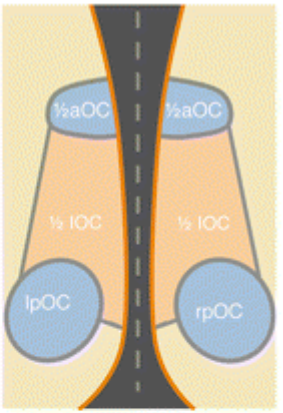
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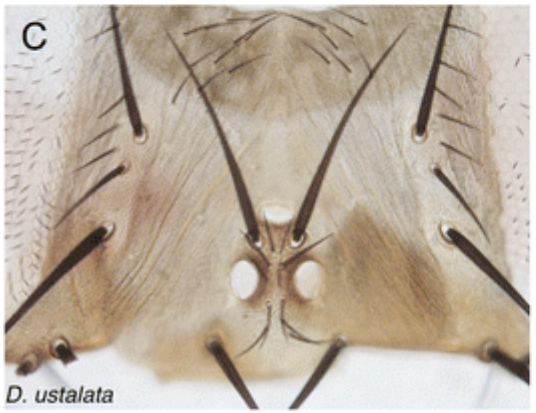
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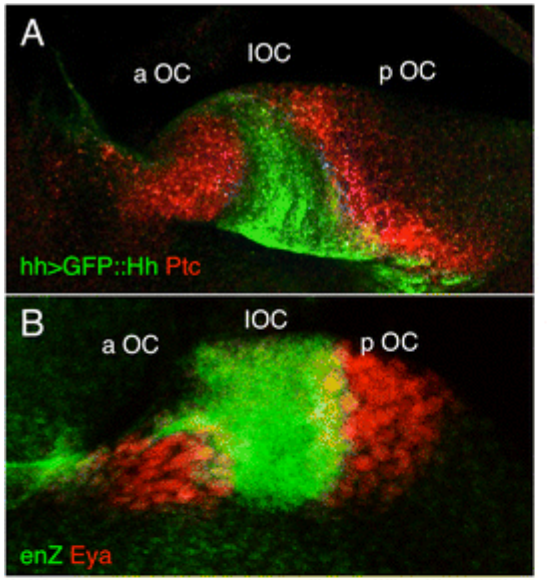
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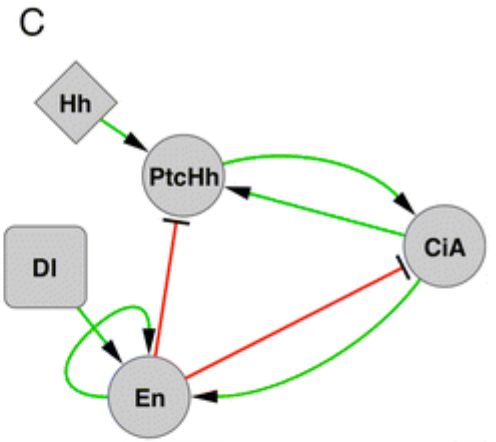
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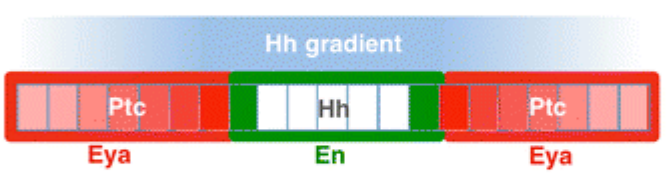
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Drosophila evolution:

Interspecific Y chromosome variation is sufficient to rescue hybrid male sterility and is influenced by the grandparental origin of the chromosomes

Araripe, L.O., Tao, Y., and Lemos, B. (2016). Heredity (Edinb) *116*, 516–522.

Here we address the contribution of 'heterospecific Y chromosomes' to fertility in hybrid males carrying a homozygous region of Drosophila mauritiana introgressed in the Drosophila simulans background.

In order to detect Y chromosome–autosome interactions, which may go unnoticed in a single-species background of autosomes, **we constructed hybrid genotypes involving three sister species**: Drosophila simulans, D. mauritiana, and D. sechellia. These engineered strains varied due to: (i) species origin of the Y chromosome (D. simulans or D. sechellia); (ii) location of the introgressed D. mauritiana segment on the D. simulans third chromosome, and (iii) grandparental genomic background (three genotypes of D. simulans).

We find complex interactions between the species origin of the Y chromosome, the identity of the D. mauritiana segment and the grandparental genetic background donating the chromosomes. Unexpectedly, the interaction of the Y chromosome and one segment of D. mauritiana drastically reduced fertility in the presence of Ysim, whereas the fertility is partially rescued by the Y chromosome of D. sechellia when it descends from a specific grandparental genotype. The restoration of fertility occurs in spite of an autosomal and X-linked genome that is mostly of D. simulans origin. **These results illustrate the multifactorial basis of genetic interactions involving the Y chromosome.** Our study supports the hypothesis that the **Y chromosome can contribute significantly to the evolution of reproductive isolation** and highlights the conditional manifestation of infertility in specific genotypic combinations.

Morphology evolution:

Measurement error in geometric morphometrics

Fruciano, C. (2016).

Dev. Genes Evol. *226*, 139–158.

Geometric morphometrics—a set of methods for the statistical analysis of shape once saluted as a revolutionary advancement in the analysis of morphology —is now mature and routinely used in ecology and evolution. However, a factor often disregarded in empirical studies is the presence and the extent of measurement error.

Here, I briefly **review common sources of error in geometric morphometrics**. I then review the **most commonly used methods to measure and account for both random and non-random measurement error, providing a worked example using a real dataset**.

Morphology evolution:

Selection on an antagonistic behavioral trait can drive rapid genital coevolution in the burying beetle, *Nicrophorus vespilloides*

Hopwood, P.E., Head, M.L., Jordan, E.J., Carter, M.J., Davey, E., Moore, A.J., and Royle, N.J. (2016).

Evolution *70*, 1180–1188.

Male and female genital morphology varies widely across many taxa, and even among populations. Disentangling potential sources of selection on genital morphology is problematic because each sex is predicted to respond to adaptations in the other due to reproductive conflicts of interest.

To test how variation in this sexual conflict trait relates to variation in genital morphology we used our previously developed artificial selection lines for high and low repeated mating rates.

We selected for high and low repeated mating rates using monogamous pairings to eliminate contemporaneous female choice and male–male competition. Male and female genital shape responded rapidly to selection on repeated mating rate. High and low mating rate lines diverged from control lines after only 10 generations of selection. We also detected significant patterns of male and female genital shape coevolution among selection regimes. We argue that because our selection lines differ in sexual conflict, these results support the hypothesis that sexually antagonistic coevolution can drive the rapid divergence of genital morphology. The greatest divergence in morphology corresponded with lines in which the resolution of sexual conflict over mating rate was biased in favor of male interests.

Morphology evolution:

The gene *cortex* controls mimicry and crypsis in butterflies and moths

Nadeau, N.J., Pardo-Diaz, C., Whibley, A., Supple, M.A., Saenko, S.V., Wallbank, R.W.R., Wu, G.C., Maroja, L., Ferguson, L., Hanly, J.J., et al. (2016).

Nature *534*, 106–110.

Here, we use fine-scale mapping with population genomics and gene expression analyses **to identify a gene, *cortex*, that regulates pattern switches** in multiple species across the mimetic radiation in *Heliconius* butterflies. *cortex* belongs to a fast-evolving subfamily of the otherwise highly conserved fizzy family of cell-cycle regulators, suggesting that it probably regulates pigmentation patterning by regulating scale cell development. In parallel with findings in the peppered moth (*Biston betularia*), our results suggest that this mechanism is common within Lepidoptera and that *cortex* has become a major target for natural selection acting on colour and pattern variation in this group of insects.

The industrial melanism mutation in British peppered moths is a transposable element

Van't Hof, A.E., Campagne, P., Rigden, D.J., Yung, C.J., Lingley, J., Quail, M.A., Hall, N., Darby, A.C., and Saccheri, I.J. (2016).

Nature *534*, 102–105.

Discovering the mutational events that fuel adaptation to environmental change remains an important challenge for evolutionary biology. The classroom example of a visible evolutionary response is industrial melanism in the peppered moth (*Biston betularia*): the replacement, during the Industrial Revolution, of the common pale *typica* form by a previously unknown black (*carbonaria*) form, driven by the interaction between bird predation and coal pollution. The *carbonaria* locus has been coarsely localized to a 200-kilobase region, but the specific identity and nature of the sequence difference controlling the *carbonaria–typica* polymorphism, and the gene it influences, are unknown. Here we show that the mutation event giving rise to industrial melanism in Britain was the **insertion of a large, tandemly repeated, transposable element into the first intron of the gene *cortex***. Statistical inference based on the distribution of recombined *carbonaria* haplotypes indicates that this transposition event occurred around 1819, consistent with the historical record.

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Cis-regulatory evolution:

Mechanisms of transcription factor evolution in Metazoa

Schmitz, J.F., Zimmer, F., and Bornberg-Bauer, E. (2016).
Nucleic Acids Res. *44*, 6287–6297.

Transcriptions factors (TFs) are pivotal for the regulation of virtually all cellular processes, including growth and development. Expansions of TF families are causally linked to increases in organismal complexity. **Here we study the evolutionary dynamics, genetic causes and functional implications of the five largest metazoan TF families.** We find that family expansions dominate across the whole metazoan tree; however, some branches experience exceptional family-specific accelerated expansions. Additionally, we find that such expansions are often predated by modular domain rearrangements, which spur the expansion of a new sub-family by separating it from the rest of the TF family in terms of protein–protein interactions. This separation allows for radical shifts in the functional spectrum of a duplicated TF. We also find functional differentiation inside TF sub-families as changes in expression specificity. Furthermore, accelerated family expansions are facilitated by repeats of sequence motifs such as C2H2 zinc fingers. We quantify whole genome duplications and single gene duplications as sources of TF family expansions, implying that some, but not all, TF duplicates are preferentially retained. We conclude that trans-regulatory changes (domain rearrangements) are instrumental for fundamental functional innovations, that cis-regulatory changes (affecting expression) accomplish wide-spread fine tuning and both jointly contribute to the functional diversification of Tfs.

Drosophila behavior:

Experimental evolution under hyper-promiscuity in *Drosophila melanogaster*

Perry, J.C., Joag, R., Hosken, D.J., Wedell, N., Radwan, J., and Wigby, S. (2016).
BMC Evol. Biol. *16*, 131.

Here, we use a genetic manipulation of mating frequency in *Drosophila melanogaster* to create a novel, highly promiscuous mating system. **We generated *D. melanogaster* populations in which flies were deficient for the sex peptide receptor (*SPR*) gene – resulting in *SPR*- females that mated more frequently – and genetically-matched control populations, and allowed them to evolve for 55 generations.** At several time-points during this experimental evolution, we assayed behavioural, morphological and transcriptional reproductive phenotypes expected to evolve in response to increased population mating frequencies.

We found that males from the high mating frequency *SPR*- populations evolved decreased ability to inhibit the receptivity of their mates and decreased copulation duration, in line with predictions of decreased per-mating investment with increased sperm competition. Unexpectedly, *SPR*- population males also evolved weakly increased sex peptide (*SP*) gene expression. Males from *SPR*- populations initially (i.e., before experimental evolution) exhibited more frequent courtship and faster time until mating relative to controls, but over evolutionary time these differences diminished or reversed.

In response to experimentally increased mating frequency, *SPR*- males evolved behavioural responses consistent with decreased male post-copulatory investment at each mating and decreased overall pre-copulatory performance. The trend towards increased SP gene expression might plausibly relate to functional differences in the two domains of the SP protein. Our study highlights the utility of genetic manipulations of animal social and sexual environments coupled with experimental evolution.

Drosophila behavior:

Fighting experience affects fruit fly behavior in a mating context.

Teseo, S., Veerus, L., and Mery, F. (2016). Naturwissenschaften *103*, 38.

Here, we use *Drosophila melanogaster* to investigate the effects of conflict-induced behavioral modifications on male mating behavior.

In *D. melanogaster*, males fight for territories and experience a strong winner-loser effect, meaning that winners become more likely to win subsequent fights compared to losers, who continue to lose.

In our protocol, males were tested for courtship intensity before and after fighting against other males.

We show that male motivation to copulate before fights cannot predict the fight outcomes, but that, afterwards, losers mate less than before and less than winner and control males. Contrarily, winners show no differences between pre- and post-fight courtship intensity, and do not differ from control males. This suggests that the physiological modifications resulting from fight outcomes indirectly affect male reproductive behavior.

Mutation rate:

Purifying selection shapes the coincident SNP distribution of primate coding sequences

Chen, C.-Y., Hung, L.-Y., Wu, C.-S., and Chuang, T.-J. (2016b).

Sci Rep *6*, 27272.

Genome-wide analysis has observed an **excess of coincident single nucleotide polymorphisms (coSNPs) at human-chimpanzee orthologous positions, and suggested that this is due to cryptic variation in the mutation rate**. While this phenomenon primarily corresponds with non-coding coSNPs, the situation in coding sequences remains unclear.

Here we calculate the observed-to-expected ratio of coSNPs ($coSNP_{O/E}$) to estimate the prevalence of human-chimpanzee coSNPs, and show that the excess of coSNPs is also present in coding regions. Intriguingly, $coSNP_{O/E}$ is much higher at zero-fold than at nonzero-fold degenerate sites; such a difference is due to an elevation of $coSNP_{O/E}$ at zero-fold degenerate sites, rather than a reduction at nonzero-fold degenerate ones.

These trends are independent of chimpanzee subpopulation, population size, or sequencing techniques; and hold in broad generality across primates. We find that this discrepancy cannot fully explained by sequence contexts, shared ancestral polymorphisms, SNP density, and recombination rate, and that $coSNP_{O/E}$ in coding sequences is significantly influenced by purifying selection. We also show that selection and mutation rate affect $coSNP_{O/E}$ independently, and coSNPs tend to be less damaging and more correlated with human diseases than non-coSNPs. These suggest that coSNPs may represent a “signature” during primate protein evolution.

Mutation rate:

High-specificity detection of rare alleles with Paired-End Low Error Sequencing (PELE-Seq)

Preston, J.L., Royall, A.E., Randel, M.A., Sikkink, K.L., Phillips, P.C., and Johnson, E.A. (2016).

BMC Genomics *17*, 464.

Polymorphic loci exist throughout the genomes of a population and provide the raw genetic material needed for a species to adapt to changes in the environment. The minor allele frequencies of rare Single Nucleotide Polymorphisms (SNPs) within a population have been difficult to track with Next-Generation Sequencing (NGS), due to the high error rate of standard methods such as Illumina sequencing.

We have developed a method of rare allele detection that mitigates both sequencing and PCR errors, called PELE-Seq. PELE-Seq was evaluated using control *E. coli* populations and was then used to compare a wild *C. remanei* population to a lab-adapted population. The PELE-Seq method is ideal for investigating the dynamics of rare alleles in a broad range of reduced-representation sequencing methods, including targeted amplicon sequencing, RAD-Seq, ddRAD, and GBS. PELE-Seq is also well-suited for whole genome sequencing of mitochondria and viruses, and for high-throughput rare mutation screens.

Functional genomics:

Editing Transgenic DNA Components by Inducible Gene Replacement in *Drosophila melanogaster*

Chun-Chieh Lin, Christopher J. Potter

Genetics August 1, 2016 vol. 203 no. 4 1613-1628; DOI: 10.1534/genetics.116.191783

We leveraged gene conversion to develop a method for genomic editing of existing transgenic insertions in *Drosophila melanogaster*. The clustered regularly-interspaced palindromic repeats (CRISPR)/Cas9 system is used in the **h**omology **a**ssisted **C**RISPR **k**nock-in (HACK) method to induce double-strand DNA breaks in a *GAL4* transgene, which is repaired by a single-genomic transgenic construct containing *GAL4* homologous sequences flanking a *T2A-QF2* cassette. With two crosses, this technique converts existing *GAL4* lines, including enhancer traps, into functional *QF2* expressing lines. We used HACK to convert the most commonly-used *GAL4* lines (labeling tissues such as neurons, fat, glia, muscle, and hemocytes) to *QF2* lines. We also identified regions of the genome that exhibited differential efficiencies of HDR. The HACK technique is robust and readily adaptable for targeting and replacement of other genomic sequences, and could be a useful approach to repurpose existing transgenes as new genetic reagents become available.

Human evolution:

Homo floresiensis-like fossils from the early Middle Pleistocene of Flores

van den Bergh, G.D., Kaifu, Y., Kurniawan, I., Kono, R.T., Brumm, A., Setiyabudi, E., Aziz, F., and Morwood, M.J. (2016).

Nature *534*, 245–248.

"**Hobbit" relatives found after ten-year hunt**

Callaway, E. (2016).

Nature *534*, 164–165.