How do Drosophila evolve two types of sperm?

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Abstract: Sexual selection drives numerous animal species to independently evolve infertile parasperm that facilitate, provision or compete with the fertile eusperm. Such sperm heteromorphism provides a paradigm for understanding the evolution of cell-level novelty and altruism, but the underlying genetic and developmental programs remain uncharacterized. Here we use Drosophila pseudoobscura (Dpse) as a model and resolve its two lineages of parasperm that segregate by their spatially-resolved single-cell transcriptomes from the eusperm class and sperm of D. melanogaster (Dmel). Transcribed Dpse-specific new genes, including de novo genes originated from non-coding sequences are underrepresented in early spermatocytes and on the X chromosome due to meiotic X-inactivation; but are enriched in late meiotic cells and known transcription regulators in spermatogonia, as well as those that are differentially expressed between eusperm and parasperm. We identify many candidate upstream transcription factors in spermatogonia that probably orchestrate an expression gradient between the three sperm lineages in many downstream genes functioning in mitochondrial morphogenesis, sperm individualization and tail elongation etc. Our results together support a disproportionately important role of new genes in evolution of new sperm morphs. These low-cost parasperm probably shield the eusperm from the hostile female reproductive tract until fertilization.

Keywords: Drosophila;sperm heteromorphism;evolution

The origin and evolution of Chinese oak silkworm, *Antheraea pernyi*

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Abstract: The domestication event of Chinese oak silkworm *Antheraea pernyi* that is the most famous among wild silkworms worldwide occurred in China;however, the two key issues on the origin and evolution of the domestic type of*A. pernyi* (ApD) including the wild ancestor(wild type of *A. pernyi*, ApW) and domestication site remain controversial. The mitochondrial gene (genome) was first used to compare the domesticated samples and light-trapped wild samples.The phylogenetic analysis based on partial COI gene corresponding to the DNA barcode from 230 samples/sequences of *Antheraea* genus identified that ApWis indeed distributed into Southern China, while the so called "*A. roylei*" is extremely scarce.The mean Kimura-2-Parameter genetic distance is 0.0168 ± 0.0045 between ApD and ApW population, confirming the presence of ApW. Further phylogenetic analysis based on the mitochondrial genome also suggested that only one geographically distinct event occurred in domestication of ApD,and that the domestication event of ApD might occur in Henan Province,rather than widely accepted Shandong Province. Finally, we provide a telomere-to-telomere genome assembly for ApW,named ApW-SYAU. K-mer analysis based on Illumina short reads revealed a high level of heterozygosity of 2.8% for ApW.The near-complete genome of ApW-SYAU is 694.41Mb long with contig N50 of 13.2Mb,and 49 chromosomes.The ApW-SYAU genome contains 55.07% of repetitive sequences, with a total length of 382,409,190 bp,mainly the Helitron and Long interspersed nuclear element (LINE) families. Depending on the coverage of there peats, 87 telomeres were identified, and 34 chromosomes with zero gap were constructed. In total, 24,783 protein-coding genes were identified in the ApW-SYAU genome. Arootedphylo gene tic inference based on 1433 single-copy genes confirmed the close relationship between ApW and its domestic counterpart ApD,and MCMC Tree estimated that ApD diverged fromits wild ancestor ApW about3.22~25.87Mya, far greater than that between the domestic silk moth (B.mori)and its wild ancestor (B.mandarina) with a divergence time of 0.003985~0.004305 Mya. Our work provides insight into evolutionary patterns between wild and domestic type of A. pernyi.

Keywords: Antheraea pernyi; wild ancestor; domestication site; mitochondrial genome; telomere-to-telomere genome

The bright and dark sides of fireflies

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Abstract: Fireflies were believed to originally evolve their novel bioluminescence as warning signals to advertise their toxicity to predators, which was later adopted in adult mating. Although the evolution of bioluminescence has been investigated extensively, the warning signal hypothesis of its origin has not been tested. In this study, we test this hypothesis by systematically determining the presence or absence of firefly toxin lucibufagins (LBGs) across firefly species and inferring the time of origin of LBGs. We confirm the presence of LBGs in the subfamily Lampyrinae, but more importantly, we reveal the absence of LBGs in other lineages, including the subfamilies of Luciolinae, Ototretinae, and Psilocladinae, two incertae sedis lineages, and the Rhagophthalmidae family. Ancestral state reconstructions for LBGs based on firefly phylogeny constructed using genomic data suggest that the presence of LBGs in the common ancestor of the Lampyrinae subfamily is highly supported but unsupported in more ancient nodes, including firefly common ancestors. Our results suggest that firefly LBGs probably evolved much later than the evolution of bioluminescence. We thus conclude that firefly bioluminescence did not originally evolve as direct warning signals for toxic LBGs and advise that future studies should focus on other hypotheses. Moreover, LBG toxins are known to directly target and inhibit the α subunit of Na+, K+-ATPase (ATPα). We further examine the effects of amino acid substitutions in firefly ATPα on its interactions with LBGs. We find that ATPα in LBG-containing fireflies is relatively insensitive to LBGs, which suggests that target-site insensitivity contributes to LBG-containing fireflies' ability to deal with their own toxins.

Keywords: firefly;bioluminescence;toxin

Comparative genomics of parasitoid wasps

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Abstract: Parasitoid wasps are parasitic insects with high species diversity and have played a pivotal role in biological control and evolutionary studies. With the significant advancements insequencing technologies, both the quality and quantity of genomes have been improved, making hundreds of parasitoid wasp genomes available and laying the foundation for large-scale comparative genomic research. In this study, based on our sequencing of over 20 parasitoid wasp genomes, we conducted a series of comparative genomic studies to provide insights into the genomic basis of several key traits, including genome size evolution, body size reduction and yolk loss. Our research highlights the crucial role of comparative genomic studies in exploring the evolution of parasitoid wasps, particularly in understanding the mechanisms underlying diversity evolution and the adaptive evolution of parasitic traits.

Keywords: Genome sequencing, Comparative genomics, Parasitoid wasps, Evolution

Temporal genomics in Hawaiian crickets reveals compensatory intragenomic coadaptation during adaptive evolution

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Abstract: Theory predicts that compensatory genetic changes reduce negative indirect effects of selected variants during adaptive evolution, but evidence is scarce. Here, we test this in a wild population of Hawaiian crickets using temporal genomics and a high-quality chromosome-level cricket genome. In this population, a mutation, *flatwing*, silences males and rapidly spread due to an acoustically-orienting parasitoid. Our sampling spanned a social transition during which *flatwing* fixed and the population went silent. We find long-range linkage disequilibrium around the putative *flatwing* locus was maintained over time, and hitchhiking genes had functions related to negative *flatwing*-associated effects. We develop a combinatorial enrichment approach using transcriptome data to test for compensatory, intragenomic coevolution. Temporal changes in genomic selection were distributed genome-wide and functionally associated with the population’s transition to silence, particularly behavioural responses to silent environments. Our results demonstrate how ‘adaptation begets adaptation’; changes to the sociogenetic environment accompanying rapid trait evolution can generate selection provoking further, compensatory adaptation.

Keywords: adaptation genomics;host-parasite coevolution;negative pleiotropy;secondary adaptation;trait loss

A Large Number of Animal microRNAs have Long recursors

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Abstract: MicroRNAs (miRNAs) are well-established post-transcriptional regulators of gene expression, typically generated from short, stem-loop precursors. In this study, we discovered a novel class of miRNAs, termed long precursor miRNAs(LP-miRNAs), in multiple insect species. To identify LP-miRNAs, we developed a new software tool, LPmiPred, and applied it to small RNA sequencing data from 35 species. We identified 42,812 LP-miRNAs,revealing their wide spread occurrence across vertebrates, worms, and insects. Focusing onlpmiR-12, a highly expressed LP-miRNA with a 266–556 nt branched terminal loop, we demonstrated its functional role in *Plutella xylostella*. CRISPR/Cas9-mediated knockout oflpmiR-12 disrupted proboscis development, affirming that LP-miRNAs perform criticalregulatory functions similarto canonical miRNAs. Our findings challenge conventional miRNA biology, highlighting the prevalence and importance of LP-miRNAs in animals,and provide new avenues for understanding the complexity of gene regulation through miRNAs.

Keywords: miRNA; Long precursor; Identification; Animals

Genetic mechanism underlying color polymorphism in the short-headed bumble bee, Bombus breviceps

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Abstract: The short-headed bumble bee Bombus breviceps (Hymenoptera: Apidae) is a key pollinator in Southern Asia, which exhibits at least 10 color variations across different geographic populations, making it an ideal model for studying trait adaptive evolution. However, the molecular mechanisms underlying the color polymorphism of this species remain unknown. This study investigates the molecular basis of the orange-black color dimorphism on the fifth abdominal tergite (T5). Genetic hybridization analysis indicates that the orange-black dimorphism is controlled by a single genetic locus, with the orange phenotype being dominant. Whole-genome association analysis localized this locus between the Hox genes abd-A and Abd-B, which determine the developmental fate of the insect's segments. Genotyping narrowed the locus to a 1.4 kb region, ~25 kb downstream of Abd-B. The locus associated with driving the red-black abdominal variation in the North American distant-related bumblebee species, Bombus melanopygus, is located in the Hox region, but at different positions. Transcriptome analysis, gene expression profiling, and RNA interference experiments indicate that the spatiotemporally specific expression of Abd-B and a novel upstream long non-coding RNA (lncRNA) during the critical period of color differentiation may drive the orange-black transition by inducing homeotic transformation in late development. Downstream melanin synthesis pathway genes (Ddc3, yellow-d, black, etc.) may also contribute to hair color differentiation. In conclusion, this study identifies the Hox region as a genomic hotspot for color pattern evolution in Bombus and demonstrates how pleiotropic developmental loci can drive adaptive radiation in nature, generating complex trait diversity.

Keywords: Bumble bee;color polymorphism;Hox;non-coding RNA

Does the origin of novel phenotypes and their integration into complex systems evolve predictably?

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Abstract: The evolution of a novel, big headed, soldier caste has evolved multiple times independently within ants. Despite being wingless, the soldier caste larvae develop wing imaginal disc during larval development in the *Pheidole hyatti*. Rajakumar *et al*. 2018 discovered that this rudiment has been repurposed to function in generating the disproportionately large head of the soldier caste, furthermore, the inhibitory pheromone (cuticular extract) produced by adult soldiers suppresses the production of soldiers through suppressing the growth of the rudimentary wing disc. We use the *Camponotus floridanus*, a species with independently evolved soldier caste, to ask if the origin and regulation of the novel soldier caste in an independently evolved complex worker caste systems evolve in parallel or convergently? If the rudimentary wing disc is repurposed to function in generating the disproportionately large head in the soldier caste, and it is regulated by inhibitory pheromone then it’s parallel evolution, if not then it’s convergent evolution. Using RNAi knock down of a key gene in the wing disc growth network, we significantly reduce the head to body ratio compared to the control. Like P.hyatti, the vestigial wing disc of the *C.floridanus* had been co-opted to regulate the head to body scaling in soldier development, therefore the novel soldier castes generated though parallel evolution. But application of the cuticular extract produces no discernable effect. However, we find the trophallactic liquid extracted from adult soldiers inhibits wing disc growth of larvae. We show the soldier caste in *C.floridanus* is not regulated through inhibitory pheromone, but rather, through inhibitory effects from adult soldier trophallactic liquid, and therefore the incorporation and regulation of this novel caste is convergent. We propose that while novel complex phenotypes may evolve using similar developmental processes, the integration of novel phenotypes into existing regulatory interactions or complex systems can be different.

Keywords: Development, Parallel/convergent evolution, Allometry, Ant, Social insects

The pigment transporter Redboy confers programmed body colour transition in orchid mantises

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Abstract: Programmed phenotypic transition is prevalent throughout the tree of life, yet the concrete mechanisms that underpin this phenomenon are poorly understood. The orchid mantis (*Hymenopus coronatus*, Mantodea) is a model study system for programmed body colour transitions that displays a prominent black-red body colour in first-instar nymphs, then switches to a flowery white body colour in later-instar nymphs. Here we reveal that this body colour transition is achieved by the simultaneous excretion of decarboxylated-xanthommatin (red pigment) and the accumulation of uric acid (white pigment) in the epidermis during the first moult. This change in pigmentation is associated with a novel subtype of ABCG pigment transporter that we call 'Redboy' in Polyneoptera, which is upregulated by insect steroid hormone (ecdysone) during the first moult of orchid mantises. RNAi assay and pigment analyses show that Redboy functions together with the co-transporter White, exporting red pigments from and concurrently importing white pigments into the epidermal cells. Spectral reflectance analyses and predation experiments reveal that Redboy-conferred programmed body colour transition enhances predator avoidance during the first instar, and both prey attraction and predator avoidance in later instars. Our findings clarify how gene family evolution and hormone regulation coordinate programmed phenotypic transition and promote ecological adaptation in orchid mantises.

Keywords: Programmed body colour transition;Orchid mantis;Molecular basis;Ecological significance

Genetic mechanism underlying pollination and ecological adaptation in *Megachile rotundata*

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Abstract: The leafcutter bee *Megachile rotundata* is the world's most intensively managed solitary bee, owing to its easy manipulation and high pollination efficacy. Here, a high-quality chromosome-level *M. rotundata* genome, covering 280.68 Mb is presented. A total of 10701 genes are predicted, of which 93.06% are functionally annotated. Based on the new genome assembly, transposable elements, non coding RNAs, as well as gene families associated with pollination biology and ecological adaptation are systematically characterized. Comparative genomic analysis shows a notable expansion of *Toll* gene family but the contraction of detoxification genein *M. rotundata* genome. Surprisingly, these expanded *Toll-1* genes andtheir downstream genes display abundant mRNA levels in diapausing prepupae.Additionally, diapausing prepupae show significantly upregulated expression of antimicrobial peptide genes and ahigher survival rate after *Escherichia coli* exposure compared to nondiapausing prepupae, indicatingan enhanced immune response during *M. rotundata*diapause. The *M.rotundata* genome provides an important foundation for understanding itsecological adaptation and optimizing its exception alpollination efficiency in the future.

Keywords：diapause, evolution, genome, insect, toll receptors

A-to-I RNA editing in insects: what, how, & why?

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Abstract: AADAR-mediated A-to-I RNA editing is a post-transcriptional modification that introduces A-to-G variations in RNAs. Insects usually have thousands of RNA editing sites per species and hundreds of them are located in CDS, where the nonsynonymous RNA editing events are positively selected. An ultimate question is: what is the advantage of RNA editing compared to DNA mutations? We find that RNA editing is dynamic in response to development, caste differentiation, and environmental cues, resolving the pleiotropic effects of DNA mutations. Temperature-sensitive RNA recoding sites are frequently found in essential ion channels. During evolution, editing sites might derive from ancestral uneditable codons, and then those genomic adenosines are selectively maintained, highlighting the necessity of RNA editing. In summary, in contrast to DNA mutations, RNA editing is another route to complexity that facilitates the organisms rapidly adapt to variable environments.

Keywords: A-to-I RNA editing; nonsynonymous; dynamic; selectively maintained; adaptive

Telomere-to-Telomere Genome Datasets for Pests as a Foundation for Future Pest Management: A Case Study on Bactrocera dorsalis

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Abstract: Genomic approaches are increasingly vital for pest control research, enabling the discovery of molecular targets, understanding resistance mechanisms, and developing biological control strategies. High-quality reference genomes are foundational to these efforts. The advent of third-generation sequencing technologies has ushered in the telomere-to-telomere (T2T) era, addressing challenges in assembling highly repetitive and structurally complex regions of the genome. In this study, we successfully assembled a high-quality telomere-to-telomere (T2T) genome for Bactrocera dorsalis, a representative model for studying dipteran pests. The assembly resolves previously inaccessible regions, including centromeric and pericentromeric regions, sex chromosomes, and structurally complex loci with tandemly duplicated genes, such as odorant receptor gene clusters. These results provide detailed structural insights into repetitive and functionally critical regions of the genome, laying a solid foundation for advancing Dipteran genomics and developing future pest management strategies.

Keywords: Bactrocera dorsalis; T2T genome; pest management

Gene drive-based population modification and suppression of Anopheles stephensi

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Abstract: Gene drive alleles bias inheritance in their favor, allowing them to quickly spread throughout a population after a small release of transgenic individuals. They could combat vector-borne diseases such as malaria from mosquitoes by rapidly spreading a cargo gene that blocks pathogen transmission or by directly suppressing vector populations. However, CRISPR gene drives often generate resistance alleles when DNA cleavage is repaired by end-joining instead of homology-directed repair, which impedes the spread of the drive. To overcome resistance allele formation, we combined several methods in *Anopheles stephensi* mosquitoes. Computational modeling predicts successful disease elimination for suppression drives despite the "chasing" phenomenon that causes chaotic, long-term persistence of both drive and wild-type alleles. We've also constructed modification drives that can be confined to a specific target population. Additional self-limiting drives enable SIT-like suppression with substantially reduced release requirements.

Keywords: gene drive;Anopheles stephensi;CRISPR

Phylogenomic Insights into the Origin and Evolution of the only highly ant-mimicking stink bug Pentamyrmex spinosus

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Abstract: Myrmecomorphy, the mimicry of ants in morphology and behavior, has evolved many times independently across arthropods, but remains exceptionally rare in Pentatomoidea (stink bugs). *Pentamyrmex spinosus* Rider & Brailovsky, 2014 is the sole exception that exhibits a high degree of myrmecomorphy during both the adult and nymphal stages in stink bugs. Here, we integrate phylogenomics, divergence time estimation, and ecological observations to investigate its origin and the possible evolutionary road. Through field observations, we obtained the biological information of this species for the first time, *P. spinosus* inhabits bamboo habitats and coexists with the spiny ant species *Polyrhachis dives* (Smith, 1857). *Pentamyrmex spinosus* has only been observed feeding on bamboo leaves, whereas predation on ant eggs, larvae, pupae, adults, or other arthropods has not been documented in either the wild or laboratory conditions. We reconstructed phylogenetic relationships of Pentatomoidea using low-coverage genomic data, from 106 species (50 newly sequenced). The dated phylogenetic analyses indicated *P. spinosus* as the sister group to the grass-feeding Phyllocephalinae, with divergence at ~42.7 Ma (95% HPD: 52.6–33.2 Ma). This timing coincides with the radiation of the ant genus *Polyrhachis* (42.0–33.0 Ma) and the early diversification of bamboos (66.9–24.9 Ma). Our results suggest the rapid diversification of *Polyrhachis* ants and bamboo probably drove the evolution of *P. spinosus*, enabling niche adaptation. Additionally, we recovered six robust clades within Pentatomoidea, clarifying contentious systematic relationships of some families (eg. Urostylididae + Saileriolidae, Canopidae + Megarididae, Acanthosomatidae + Lestoniidae, and Serbaninae + Phloeidae). This study illuminates the interplay between ecological interactions and morphological evolution, providing a framework for understanding myrmecomorphy in insects and higher classification of Pentatomoidea.

Keywords: phylogeny;true bugs;Pentatomidae;myrmecomorphy;divergence time;ant;bamboo

Chromosome-scale genome dynamics reveal signatures of independent haplotype evolution in the ancient asexual mite Platynothrus peltifer

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Abstract: Even if asexuality is regarded as an evolutionary dead-end, some "ancient asexual scandals" have persisted and diversified over time, including several species of oribatid mites. Very little is known about the genomic footprints of such long-term evolution in the absence of sex. Identifying such signatures will help to understand why some asexuals can escape the dead-end fate and to identify the benefits of sex vice-versa. However, the tiny body size and exocrine glands make low-input DNA a bottleneck for genomic studies on oribatid mites. Here, we overcame this obstacle and generated a phased, high-quality reference genome of the parthenogenetic oribatid mite *Platynothrus peltifer* based on a single individual, using novel long-read and linked-read approaches. We analyzed allelic sequence divergence, differentially expressing alleles (DEA), transposable element landscape, and the amount of horizontally transferred genes. Between the two haplotypes, 10% of the single-copy genes showed DEA. Furthermore, the *Ka/Ks* value of DEA was relatively higher than that of equally expressing genes. Moreover, Horizontal Gene Transfer (HGT) could supply the substrate for novelty via modulating gene regulation in these mites. Multiple lines of evidence indicate conservation of one haplotype copy and relaxed selection in the other for the ancient asexual lineages. Overall, generating this high-quality reference genome enabled the identification of signatures of evolutionary innovation, which helps to understand why some asexuals can escape the dead-end fate and reveals the advantages of sex.

Keywords: Asexual evolution;Parthenogenesis;Genome assembly;Meselson effect;Differentially expressing alleles;Horizontal Gene Transfer

Evolutionary Analysis of Olfactory Receptor Genes Across Metazoan Lineages

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Abstract: The olfactory system is critical for organismal survival and reproduction. Mammals (deuterostome representatives) and insects (protostome representatives) are evolutionarily advanced groups. Their olfactory receptors (ORs) mediate odor perception yet belong to structurally distinct gene families. The evolutionary divergence between deuterostome and protostome ORs remains unclear. This study selected 166 representative species, which spanning major evolutionary branches of Metazoa, and systematically identified 16,460 OR genes. Sequence analyses revealed significant divergence between vertebrate and insect ORs. Crucially, 134 homologous sequences of vertebrate ORs were detected in 53 insect genomes (77.9% of analyzed insects). Structural analyses showed insect OR structures differ markedly from early metazoan ORs, but exhibit higher structural similarity to cnidarian ORs relative to vertebrate ORs. Integrated evidence confirms post-Insecta divergence independent origination of insect ORs, with substantial sequence divergence from vertebrate ORs. Cnidarian ORs exhibit structural convergent similarities with insect ORs, suggesting that cnidarians once possessed but subsequently lost analogous structural evolutionary trajectories.

Keywords: Olfactory receptors;Evolution;Metazoa;Insect;Structural analyses

Landscape of structural variants reveals insights for local adaptations in the Asian corn borer

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Abstract: Capturing the genetic diversity of different wild populations is crucial for unraveling the mechanisms of adaptation and establishing links between genome evolution and local adaptation. Asian corn borer (ACB) moth has undergone natural selection during its adaptative evolution. However, structural variants (SVs), which play significant roles in these adaptation processes, have not been previously identified. Here, we constructed a muti-assembly graph pan-genome to highlight the importance of SVs in local adaptation. Our analysis revealed that the graph pan-genome contained 176.60 Mb (~37.33%) of unique sequences. Subsequently, we performed an analysis of expression quantitative trait loci to explore the impact of SVs on gene expression regulation. Notably, through quantitative trait locus (QTL) mapping analysis, we identified the FTZ-F1 gene as a potential candidate gene associated with the traits of larval development rate. Together, we explored the impact of SVs on the local adaptation of pests, therefore, facilitating accelerated pest management strategies.

Keywords: Graph pan-genome; structural variants; local adaptation; expression quantitative trait loci; Asian corn borer