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Insect Mitochondrial Genomics: A Decade of Progress

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Abstract

The past decade has seen the availability of insect genomic data explode, with mitochondrial (mt) genome data seeing the greatest growth. The widespread adoption of next-generation sequencing has solved many earlier methodological limitations, allowing the routine sequencing of whole mt genomes, including from degraded or museum specimens and in parallel to nuclear genomic projects. The diversity of available taxa now allows finer-scale comparisons between mt and nuclear phylogenomic analyses; high levels of congruence have been found for most orders, with some significant exceptions (e.g., Odonata, Mantodea, Diptera). The evolution of mt gene rearrangements and their association with haplodiploidy have been tested with expanded taxonomic sampling, and earlier proposed trends have been largely supported. Multiple model systems have been developed based on findings unique to insects, including mt genome fragmentation (lice and relatives) and control region duplication (thrips), allowing testing of hypothesized evolutionary drivers of these aberrant genomic phenomena. Finally, emerging research topics consider the contributions of mt genomes to insect speciation and habitat adaption, with very broad potential impacts. Integration between insect mt genomic research and other fields within entomology continues to be our field's greatest opportunity and challenge.

INTRODUCTION

Driven by steady decreases in cost and improvements to sequencing and assembly methods, insect genomics has truly flowered in the past decade (53). In the vanguard of this flood of data, mitochondrial (mt) genomes continue to be the most widely sequenced genome type in insects. For insects, mt genomes are both a source of data to address phylogenetic (e.g., 113), population genetic (e.g., 34), or adaptive evolution (e.g., 97) questions and a direct subject of research into genome architecture (e.g., 120), selection versus constraint (e.g., 101), and coevolution (139). Intensive studies in *Drosophila* have provided molecular-level tools and models of mt genome dynamics (for reviews, see 32, 70, 93) that inform these types of evolutionary studies across the whole of the Insecta. Even with the expanded availability of nuclear genomic data, mt genomes continue to have a vital place in insect evolutionary studies (10), connecting current studies to legacy sequence data sets (disproportionately mt genes) (e.g., 107), species ID tools (e.g., DNA barcodes), and genome-specific factors (e.g., sex-biased dispersal) (92). For these reasons, and for the unique insights that insect model clades can provide, research into the evolutionary dynamics of insect mt genomes, rather than being eclipsed by more data-intensive nuclear genomic systems, has bloomed in the past decade.

In 2014, I reviewed the state of insect mitochondrial phylogenomics just as next-generation sequencing methods were emerging (19). I reviewed the impact of mt genomic data on insect phylogenetics and model mt genomic systems within insects, along with predictions and questions to frame future research. In the intervening decade, the availability of insect mt genomes has increased almost 10-fold, and the taxonomic scale at which comprehensive comparisons can be made has become much finer. This review aims to revisit many of these same issues—sequencing and annotation methods, data availability, phylogenetic utility, mt genome rearrangements, and model systems—to identify progress, limitations, and profitable future research directions.

ADVANCES IN GENOME SEQUENCING AND ANNOTATION

As with the entire genomics field, the primary driver of insect mt genome data expansion in the past decade has been the wholesale adoption of next-generation sequencing (NGS) technologies. Prior to 2014, the majority of mt genomes were still sequenced using Sanger sequencing, and initially, NGS was applied to long polymerase chain reaction (PCR) amplicons (essentially a 1:1 replacement of Sanger technology). Increasingly, however, NGS methods are being applied directly to genomic DNA extracts via genome skimming (73). Direct NGS sequencing has the advantage of avoiding issues with PCR primer specificity or DNA fragmentation in suboptimal specimens such as those from museums (124) or subfossils (104). Due to its typically high copy number, mt genomes can be reliably obtained from NGS multiplexing [simultaneous sequencing of multiple specimens per lane (44)], mixed-species environmental samples [sometimes termed metabarcoding (24)], or as bycatch from reduced-representation library sequencing methods such as ultraconserved elements (UCEs) (e.g., 48) or anchored hybrid enrichment (AHE) (e.g., 90). Newer NGS platforms such as Nanopore/HiFi long-read sequencing have also been successfully applied to mt genomics, although the total amount of available data lags that generated by older pyrosequencing methods such as Illumina (128). Nanopore adaptive sequencing, which bioinformatically selects target regions during the sequencing cycle, has also been successfully applied, boosting the proportion of reads mapping to the mt genome by approximately 60 times (61). It is probably safe to assume that any future improvements to sequencing technologies will find rapid application to insect mt genomes and increase the rate at which mt genomes are sequenced.

The rapid adoption of NGS methods for insect mt genome sequencing has created a parallel proliferation of genome assembly methods. Sanger sequencing of amplicons has minimal

technical demands for assembly, achievable in virtually any desktop sequence management software (or even by eye) (18). In contrast, for NGS data with millions of reads per sample, of which only a small proportion map to the mt genome, automated assembly software is essential. Early studies typically used de novo assembly of the entire read pool, with the mt genome identified after assembly via blast searching the resulting contigs. This is computationally wasteful, but several specialist mt genome assemblers built on this approach, including Norgal (3) and MitoZ (82), which automate the identification of mt genomes from among assembled contigs. Much more widely used are targeted assembly pipelines. Most utilize a bait-and-iterate strategy whereby NGS reads are mapped to a target mt gene (the bait), then assembled, and the resulting contig is used to bait another round of read mapping (the iteration). The first such package was MitoBIM (46), with other widely used applications including NOVOPlasty (28), GetOrganelle (56), and MitoFinder (2) also utilizing this approach. Targeted assembly pipelines designed for general phylogenomics, such as aTRAM (1), similarly apply a bait-and-iterate approach and have been used for mt genome assembly. Targeted assemblers are significantly faster and require less computational resources than reference-free, de novo assemblers (3); however, they are limited by the similarity of bait sequences and so may fail for highly divergent species or those with nonstandard mt genome structures such as fragmentation (see 119). Testing the efficacy of different pipelines may be necessary to optimize assembly for projects concerning taxa with limited pre-existing mt genome data.

Improvements in genome annotation software have lagged behind those in assembly methods. The most widely used method, MITOS (30), utilizes secondary-structure covariance models to infer RNA genes and hidden-Markov models to predict protein coding genes. Other annotation packages, such as MitoZ (82) and MitoFlex (67), use similar classes of models, albeit with different training data. Start and stop codons are frequently inconsistently annotated by these methods, and as I have noted previously (18), alignment-based refinement of protein-coding genes is necessary for accurate use in downstream analyses such as phylogenetics or comparative genomics. Genome annotations reported to GenBank are also, unfortunately, rife with errors. For example, a recent phylogenetic analysis of Lepidoptera (20) found between 0 and 13 errors per genome (251 total across 58 genomes), with errors misplacing genes by up to 135 bp from the annotation of homologous positions in near relatives. Finally, there is an increasing trend to deposit mt genomes into GenBank without annotations, especially by large-scale genome sequencing projects such as the Darwin Tree of Life project (**http://darwintreeoflife.org**). While the timely release of data is commendable, genome annotation would add considerable value to these data. Consistent and reliable annotation is an ongoing impediment to the repeatable analysis of mt genome data.

INSECT MITOCHONDRIAL GENOME DATA

In the past decade, the total number of available insect mt genomes has increased by 876%, and total species diversity has increased by 790%, with annual growth rates of between 17–50% in genome number and 16–51% in species count (**Figure 1*a***). Total species diversity is now more than 5,800, or roughly 0.5% of described and 0.1% of estimated insect diversity (115, 149). Representation at higher taxonomic levels is now quite comprehensive. Complete ordinal representation was achieved in 2013 (with fleas and caddisflies the last to be sequenced), and mt genomes are now available for 73% of superfamilies[1] and 47% of families (149). Again, recent progress for these ranks has been rapid, with 25% of superfamilies and 27% of families first sequenced after 2014.

[1]This number includes equivalent subordinal groups for orders in which this rank is not used in their classification.

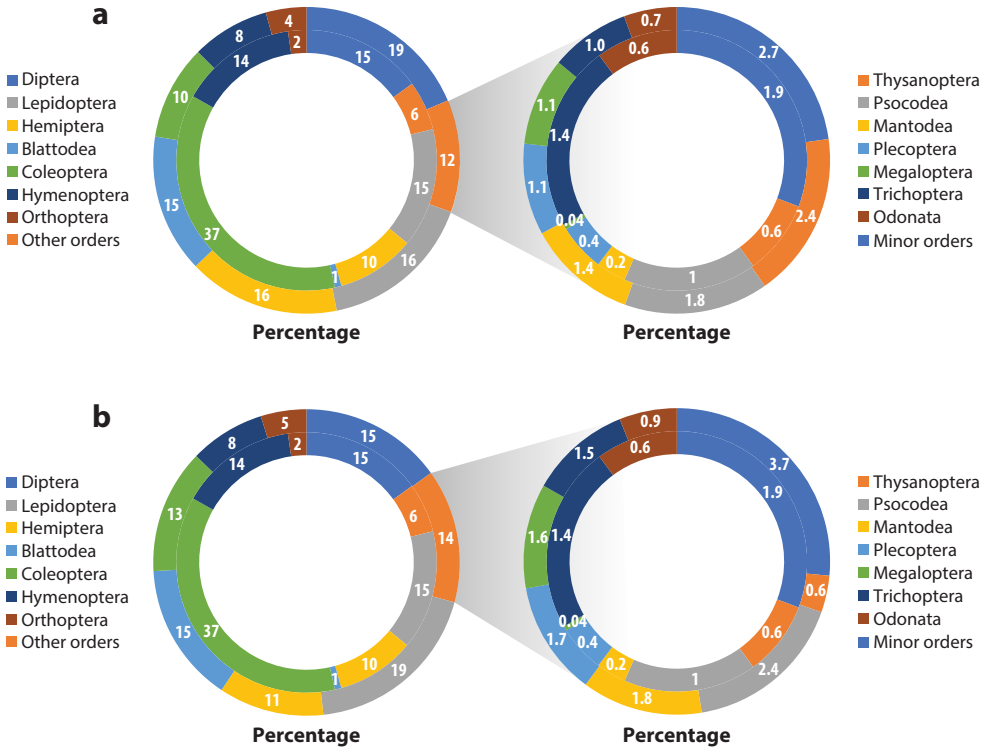

Figure 1

Ordinal-level representativeness of insect mitochondrial genome sampling to date. (*a*) Proportion of total insect mitochondrial genomes sequenced (*outer*) versus extant biodiversity (*inner*). (*b*) Proportion of total species sequenced (*outer*) versus extant diversity (*inner*). The seven most-sequenced orders are charted in the left subpanel, the next-most-sequenced orders are shown in the right subpanel (Other orders), and the remaining 14 minor orders are grouped (Minor orders).

Finally, representation of both subfamilies (up 286% to 922) and genera (up 568% to 3,225) has also increased dramatically.

Sequencing efforts across insect orders broadly parallel the species-level diversity of each order, with the five megadiverse insect orders making up five of the six most extensively sequenced orders in terms of both total available genomes and sequenced species count (**Figure 1*b***; **Supplemental Table 1**). Blattodea is the one exceptionally well-studied order with genome availability exceeding that of multiple megadiverse orders. Roach and termite mt genomes represent 14.6% (842) of total species and 12.5% (1,079) of total insect mt genomes due largely to the extensive efforts of Bourguignon and collaborators (11, 12, 48). Blattodea mt genomes are vastly in excess of the order's proportion of insect species-level diversity (approximately 0.8% of described species). Lepidoptera is also somewhat overrepresented (19% of sequenced species versus 15% of species diversity), while Hymenoptera (8% versus 14.5%) and Coleoptera (13% versus 37%) are the only orders that are significantly undersampled relative to their species diversity. Orders vary widely in the completeness of sampling at higher taxonomic levels. While each of the megadiverse orders is fairly comprehensively sequenced at the superfamily level (>80%, except Lepidoptera at 45%), considerable additional effort is needed at the family level, where completeness ranges from just 37% in Coleoptera to 61% in Hymenoptera. The mesodiverse orders (>2,000 spp.)

Supplemental Material >

include both groups that have been heavily sampled (i.e., >80% families), such as Blattodea, Plecoptera, and Neuroptera, and ones that are comparatively neglected, such as Ephemeroptera (28%), Odonata (27%), and Siphonaptera (22%). The least diverse orders tend to be very representatively sequenced (60–100% of families sampled), although this is primarily due to extant species representing only a few families.

While the rate at which mt genomes are sequenced for additional insect species is likely to continue to increase, the rate at which representatives of additional higher-level groups are sequenced is likely to slow in the coming decade. The remaining families and superfamilies are increasingly represented by monotypic, geographically restricted, and/or rarely collected species. In this regard, however, even a single dedicated study can hugely increase representation within a group. For example, Ge et al. (42) trebled our knowledge of Trichoptera, adding data for 19 additional families and increasing family coverage from 19% to 57%. Similar targeted studies of the most neglected groups (odonates, mayflies, beetles) will have the most impact on mt genome data representation in future years. Despite these knowledge gaps, the collective insect genome data set is sufficiently extensive and taxonomically representative for comparative genomic analysis across all scales, from within species to between orders.

MITOCHONDRIAL PHYLOGENOMICS OF INSECTS

Our understanding of deep-level insect phylogeny has been revolutionized in the past decade through comprehensive nuclear-genomic analysis, initially of transcriptomes (e.g., 85), and, later, via various forms of reduced genome sequencing (UCEs, AHEs, etc.). This flood of nuclear phylogenomic data allows the independent assessment of insect mt phylogenomic studies with a level of precision not previously available. Interordinal relationships inferred from mt genomes are often inconsistent with nuclear data sets (e.g., 109), especially when data sets have not been heavily modified by the exclusion of rogue taxa and/or use of corrective models (see below). Resolution within major supraordinal groups is very mixed. For relationships between winged insect groups (Odonata, Ephemeroptera, Neoptera), support is found for Metapterygota (Odonata+Neoptera) (109), Chiastomyaria (Ephemeroptera+Odonata) (142), and Palaeoptera (Odonata+Ephemeroptera) (110), despite nuclear phylogenomic data sets strongly supporting Palaeoptera (85, 102), suggesting that resolution is very sensitive to analytical artifacts. Within Polyneoptera, Forni et al. (38) found relationships strongly congruent with transcriptome phylogenies (85, 103, 136); however, outgroup choice was restricted. Resolution within the Paraneoptera has been problematic with both mt and nuclear data sets. Both major nuclear phylogenies (57, 85) failed to recover a monophyletic Paraneoptera, as Psocodea grouped with Holometabola. Mt genomic results (113) are highly sensitive to taxon choice, and no studies have included sufficient Holometabola to test the unexpected findings from transcriptome data. Finally, within Holometabola, mt phylogenomics is highly affected by compositional and mutation rate heterogeneity (106); however, removal of the fastest-evolving sites resulted in interordinal relationships largely concordant with the highly supported transcriptome-based analyses of the group (85).

Comparisons between the most representative mt and nuclear-based phylogenomic studies of insect intraordinal relationships are summarized in **Table 1**. While the limits of available data make worthwhile comparisons impossible for 5 of the smallest insect orders, the remaining 23 can be qualitatively[2] compared for topological congruence. Major subordinal relationships are congruent between data types in 16 of the 23 well-sampled orders, with Odonata, Mantodea, and

[2]Due to differences in taxon sampling between the comparison studies, algorithmic assessment of congruence is not appropriate.

Table 1 Comparison of mitochondrial genome versus nuclear data sets in resolving insect ordinal phylogenetic studies

Order	Mitochondrial	Nuclear	Comments
Archaeognatha	25	146	Highly concordant; paraphyletic Machilidae in both data sets
Zygentoma	25	NR	NA
Ephemeroptera	125	87	Highly discordant; most higher clades discordant
Odonata	110	16	Highly discordant; Anisoptera not monophyletic in mt; few interfamily nodes concordant
Zoraptera	LD	62	NA
Dermaptera	74	135	Highly discordant
Plecoptera	29	114	Superfamily resolution concordant; resolution within superfamilies varies between data sets
Orthoptera	40, 107	108	Highly concordant at interfamily nodes; Reference 108 combined mt and nuclear data sets
Mantophasmatodea	LD	98	NA
Grylloblattodea	LD	98	NA
Embioptera	LD	NR	NA
Phasmatodea	38	103	Subordinal clades concordant; relationships within Euphasmatodea highly discordant (especially placement of Phyllidae)
Mantodea	72, 78	118	Discordant at subordinal levels; significant variation between mt phylogenies
Blattodea (roaches)	12	36	Highly congruent
Blattodea (termites)	11	48	Highly congruent; Reference 48 combined mt and nuclear data sets
Thysanoptera	75, 89	13	Congruent; mt sampling limited (4/8 families); nuclear resolution poor with low loci sampling
Hemiptera	77, 113	57	Suborders congruent; Auchenorrhyncha not monophyletic in mt; relationships between Heteromorpha infraorders discordant
Psocodea	95, 141	26	Subordinal clades congruent; resolution of nonparasitic families congruent
Hymenoptera	4	9	Broadly congruent, except resolution within nonaculeate Apocrita and Symphyta
Raphidioptera	99	129	Highly concordant; mt data predominantly Inocelliidae; Sialidae undersampled
Megaloptera	55	129	Highly concordant; nuclear sampling modest
Neuroptera	111	129	Concordant at infraordinal levels; resolution of Hemerobiiform superfamilies discordant
Strepsiptera	LD	NR	NA
Coleoptera	123	17, 80	Subordinal resolution differs between mt and nuclear data sets; resolution of Polyphaga superfamilies in Reference 80 highly concordant to Reference 123, while Reference 17 differs in resolution of cerylonid series
Siphonaptera	148	150	Concordant; mt sampling very limited
Mecoptera	68	83	Concordant; Mecoptera monophyly untested in mt, not consistently found in nuclear analysis
Diptera (basal)	147	134	Highly discordant
Diptera (Brachycera)	132	6	Highly discordant
Trichoptera	42	47	Highly concordant
Lepidoptera	20, 71	59	Concordant; same nodes with poor support found in both mt and nuclear data sets

The most taxonomically comprehensive (for mitochondrial) or data-rich (for nuclear) studies were prioritized. Please see the individual studies for full details. Abbreviations: LD, limited data; mt, mitochondrial; NA, not applicable; NR, no recent studies.

Diptera being the most notable examples of high discordance. When subordinal relationships are concordant between data types, finer-scale relationships such as those between superfamilies and families are also typically concordant (10/16 orders), with exceptions where family relationships within one major subordinal clade vary (e.g., Heteroptera within Hemiptera, non-aculeate Apocrita within Hymenoptera). Conversely, while subordinal relationships within Coleoptera strongly differ between mt, Polyphaga + remainder (123) and nuclear, (Archostemata + Myxophaga) + (Adephaga + Polyphaga) (17, 80), relationships between polyphagan superfamilies are highly concordant between studies, with the placement of superfamilies like Coccinelloidea differing between nuclear studies.

These comparisons, however, do not adequately account for unevenness in analytical effort across mt phylogenomic studies and so cannot be regarded as final judgements on the mt genome's utility as a phylogenetic marker in those orders. Indeed, many of the mt phylogenomic studies that find high congruence with nuclear-based results have utilized extensive tests for sensitivity to known phylogenetic biases (e.g., nucleotide composition, rate heterogeneity, mutational saturation, rogue taxa). Compositional heterogeneity of both whole genomes and individual partitions can be readily visualized by methods such as AliGROOVE (64), which is seeing greater use in insect mt phylogenomics. Care, however, should be taken in interpreting heterogenous taxa as rogue taxa, as examples exist of both heterogenous taxa with expected topological placement (e.g., 20) and nonheterogenous taxa with wildly unexpected positions (e.g., 106). Among-site rate heterogeneity (140) is probably a more significant source of error in mt phylogenomics. Partition analysis is the most widely used approach to accounting for site heterogeneity, allowing different subsets of the mt genome to be analyzed with different models simultaneously. Automated methods for inferring optimal partitioning schemes have been implemented in standard phylogenetic inference software (e.g., IQ-Tree) (23), leading to the increased use of partitioning analysis in insect mt phylogenomics. Partitioning alone, however, is often not sufficient, and site-heterogenous models (e.g., CAT) (65) have been shown to outperform site-homogenous models for data sets with extreme compositional heterogeneity (for an example from Holometabola, see 106). It is, however, still comparatively rare for studies to test topological sensitivity by the parallel comparison of site-homogenous versus site-heterogenous models (e.g., 131). A third approach to dealing with heterogeneity has been data exclusion; the most frequently applied exclusion criteria are alignment based (e.g., GBlocks) (122). These methods, however, do not directly test site heterogeneity and are highly susceptible to alignment and annotation errors. In contrast, step-wise removal of noisy sites (observed variability sorting) (45) provides a more rigorous approach to site exclusion. Some or all of these factors may explain the discordance observed between mt and nuclear phylogenies for the orders cited above, as systematic tests for the impact of these biases have not been universal in our field.

More generally, insect mt phylogenomics needs to continue to draw methods, insights, and data from the broader phylogenetic community. Some emerging methods, such as molecular dating, have seen relatively enthusiastic application to insect mt genome data sets (e.g., 41, 89, 132). Others, such as multispecies coalescent or species-tree methods, have seen more limited uptake (but see 60). The extreme complexity of most phylogenomic data sets has led to approaches for assessing the robustness of resulting hypotheses (for a summary, see 76), many of which could be usefully applied to insect mt phylogenomics. Finally, integrated analysis of mt and nuclear data sets is still maddeningly uncommon (e.g., 48, 108), despite the fact that mt genomes can often be assembled as bycatch from most nuclear phylogenomic sequencing methods (see above). Hologenomic (mt + nuclear) approaches to insect phylogeny and evolution remain a largely unmet priority for our field.

GENOME REARRANGEMENTS AND INSECT EVOLUTION

Multiple classes of gene rearrangement have been observed in insect mt genomes, and multiple molecular mechanisms have been proposed to account for observed rearrangements. There are three basic gene rearrangement types: translocation, inversion, and inverted translocation. Each type can also be described in ways that imply the scale of rearrangement: major versus minor, local versus long range and gene-shuffling, i.e., a major translocation versus a local inversion, etc. While any two mt genomes can be compared through this framework, such comparisons have the most utility when performed within an evolutionary framework by comparison to inferred ancestral genome arrangements (e.g., 89). Three mechanistic models have been proposed for how mt gene rearrangements occur: (*a*) tandem duplication and random loss (88), (*b*) tandem duplication and nonrandom loss (66), and (*c*) recombination (33). Both tandem duplication models can only explain gene translocations (as duplicated DNA does not change coding direction, as occurs in an inversion), while recombination can explain any type of rearrangement but has a controversial history, with insect models providing some of the clearest evidence for the phenomenon (see 37, 79). Translocations are much more commonly observed than either type of inversion in both insects specifically (86) and animals in general (101). The presence of intergenic noncoding regions (e.g., 5) or pseudogenes (e.g., 138) can provide evidence in support of inferred tandem duplication–random loss events, as such nonfunctional DNA is expected during the random loss phase of this model, but in most insect taxa it is absent. Nevertheless, such events have been invoked to explain the majority of mt genome variation in insects.

While the history of many mt genome rearrangements can be reconstructed by hand, mostly for those involving few genes and/or short translocation distance (e.g., 54), a range of software has been developed to analyze more extensively rearranged mt genomes. The most widely used software, CREx/TreeREx (7), infers rearrangement events based on common intervals, i.e., sets of genes in the same order and orientation between species. Unfortunately, this software treats translocations and tandem duplication–random loss as separate events despite the majority of translocations being most parsimoniously explained by the latter. This confusion between pattern (observed genome arrangement) and process (inferred rearrangement mechanism) can potentially bias analyses of rearrangement rate (e.g., 101) or lead to misinterpretation of genome evolution (e.g., 74). Other methods, e.g., qMGR (145), utilize patterns of gene adjacency to calculate rearrangement rates. While these methods can be very useful for identifying taxa or mt regions (hot spots) with heightened rates of rearrangement, they overcount rearrangements that are conserved within a lineage. For instance, birds share a diagnostic rearrangement (84), a region that qMGR software finds to have elevated rearrangement rates (145) despite this likely being the result of a single rearrangement event in the ancestor of modern birds. Adjacency analysis also fails to account for the asymmetry between the two derived gene orders that result from each rearrangement event. The independent loss of a gene from its ancestral position (e.g., from a gene order *trnA-trnR-trnN* to *trnA-trnN* by translocation of *trnR*) in multiple lineages will produce convergent gene adjacencies, whereas the convergence of novel gene orders (e.g., a novel gene order *trnA-trnT-trnR-trnN* formed by the translocation of *trnT* into an ancestral gene order *trnA-trnR-trnN*) is much less likely if rearrangement is random (141). Many of the convergences in insect gene orders identified by Moreno-Carmona et al. (86) demonstrate this point; e.g., the gene pair *trnI-trnM* (derived from the ancestral *trnI-trnQ-trnM* gene order) is found in species from six different insect orders. At present, integration of multiple analytical approaches provides the clearest understanding of both genome rearrangement within insect taxa and rearrangement rates for individual genes.

The breadth of insect taxa with mt genome rearrangements relative to the inferred ancestral insect has grown immensely in the past decade, coincident with the vast increase in sequencing

(seek and you shall find). Species with rearranged mt genomes have now been found in 20 of the 28 orders (86). Despite increasing numbers of insect species with rearranged mt genomes being found, their distribution across insect diversity follows the same general patterns identified in 2014 (19): Most rearrangements are synapomorphic, and rearrangements are shared by clades ranging in taxonomic scale from interspecific to subordinal. Indeed, Hymenoptera is the only order where no species sequenced to date retains the inferred ancestral insect gene order (4). In all other orders, mt gene rearrangements define derived taxonomic groups. Even the lice and bark lice (Psocodea), whose mt genomes are usually extremely rearranged (see below), have one member who retains the ancestral genome order, *Prionoglaris* (95, 141). Moreno-Carmona et al. (86) have identified 24 genome rearrangements that are convergent across scales ranging from between orders to between genera in families, but this number is dwarfed by the total number of rearrangements found in insects. Recent reviews of rearrangement dynamics, including clade synapomorphies, have been provided for several orders, including Dermaptera (22), Mantodea (72), Orthoptera (40), Psocodea (excluding lice) (95), Thysanoptera (75), Hymenoptera (4), and Trichoptera (42).

Our greatly expanded diversity of insect mt genomes also allows us to revisit hypotheses for why some lineages appear to have greatly increased rates of genome rearrangement. Cameron (19) cited a correlation between heightened rearrangement rates and haplodiploidy (including paternal genome elimination) in insects. At that time, mt genome data was available for only five of the nine lineages in which haplodiploidy was then known (51). Data accumulated since 2014 further confirm this pattern, with highly rearranged mt genomes found in broader sampling of the Hymenoptera (4), Thysanoptera (75, 89), Sciaridae (126), and Aleyrodidae (112). Within the Psocodea, the discovery of paternal genome elimination in *Liposcelis* (52) suggests that this form of haplodipoloidy occurs throughout the clade Phthiraptera + Liposcelidae (parasitic and book lice) (26). While mt genome rearrangements are found in the bark lice (most of the former Psocoptera), including rearrangement of a block of six transfer RNAs and two protein-coding genes in the Psocomorpha (95), rearrangement rates are much higher in Phthiraptera + Liposcelidae, coincident with paternal genome elimination. Of the four haplodiploid lineages for which mt genome data were not available in 2014, three can now be tested; the archostematan beetle, *Micromalthus*, has yet to be sequenced. No member sequenced to date from either of the haplodiploid weevil groups, the tribes Xyleborini/Dryocoetini (13 spp. in 8 genera sequenced) and the genus *Hypothenemus* (5 spp. sequenced), have mt genome rearrangements. These weevil lineages are quite old; *Hypothenemus* is 57 ± 6 million years old (Myo), and Xyleborini/Dryocoetini is 22 ± 3 Myo (91), more than old enough to have accumulated genome rearrangements if such mutations were elevated in the group (compare to sucking lice, which are of a comparable age) (26). The final haplodiploid group, Coccoidea (scales), however, strongly matches this expectation. All coccoid species sequenced to date have at least one mt gene rearranged (137), with some species having more extreme rearrangements, such as of multigene blocks and/or inversions. Genome arrangements in Coccoidea also vary both within and between higher clades (77) as is observed in other highly rearranged groups such as lice, thrips and Hymenoptera. Haplodiploidy reflects a continuum of underlying karyotypic phenomena (8), and variation in how it manifests in individual taxa may explain the variation in the degree of mt genome rearrangement between the groups discussed above and, indeed, the absence of rearrangements in haplodiploid weevils. Any molecular basis linking haplodiploidy and mt genome rearrangements is as yet unknown.

INSECT MITOCHONDRIAL GENOME MODEL SYSTEMS

The past decade has also seen two aspects of insect mt genomes, which are nearly unique among animals, develop as model systems for a general understanding of mt genomic functions. The

first is mt genome fragmentation, the breakup of the single mt chromosome present in almost all animals into multiple chromosomes, each with a subset of genes (i.e., minichromosomes or minicircles) (105). Outside of insects, mt fragmentation is only known in a few species of jellyfish (130), nematodes (43), and rotifers (116) or in several human disease states (96). Within insects, mt fragmentation is common within parasitic lice (120) and their sister group, the booklouse *Liposcelis* (37); heteroplasmic fragmentation (the coexistence of full-sized and fragmentary mt genomes) has additionally been recorded in thrips (27) and a wasp (79). Mt fragmentation is extremely common in lice, being found in 54 of the 86 species sequenced to date; has independently evolved at least eight times (120) over taxonomic ranges from within genera (31) to a synapomorphic feature of the entire major mammal louse clade (Anoplura+Trichodectidae); and has persisted within clades for up to 60 million years (26). Similarly, within *Liposcelis*, mt structure is variable, with both fragmented and unfragmented species (four versus seven) and multiple origins of genome fragmentation in this extremely old genus (86 million years) (37, 100). Both groups provide strong evidence for mt recombination, shuffling genes between minichromosomes and driving concerted evolution between noncoding regions across each minichromosome within individuals (37, 39, 119).

Within lice, mt fragmentation is associated with relaxed selection on protein-coding genes (*dN/dS* ratios approach 1.0) and a reduction in AT compositional bias, potentially due to reduced replication time (120, 121). Louse clades with fragmented mt genomes also appeared to have elevated rates of gene rearrangement, notably including changes in gene composition of minichromosomes between different species within a genus (31, 39, 119), whereas rearrangements are limited or absent within louse genera that retain a single mt chromosome (31, 121). In contrast, *Liposcelis* had only mildly relaxed selection relative to other Psocoptera, and species with fragmented mt genomes did not have significantly more rearrangements than unfragmented species (37). This seeming contradiction may be due to the differences in the scale of fragmentation in the two groups: 2–3 mt minichromosomes in fragmented *Liposcelis* species versus >10 in the majority of parasitic lice with fragmented mt genomes; i.e., all of these phenomena are proposed to increase as fragmentation becomes more extreme. Feng et al. (37) and Sweet et al. (120) therefore independently concluded that fragmentation was a consequence of other factors that reduced purifying selection and proposed that similar models drive the fixation of fragmented mt genomes within lineages. The repeated evolution of mt fragmentation across lice and near relatives, combined with variation in chromosome count and size in extant taxa, provides an excellent system for testing these evolutionary scenarios and comparing them against nonanimal mt genomic systems, where such chromosomal variation is far more common (105).

The second insect mt genomic model that has emerged is the duplication of control regions found in thrips (Thysanoptera) (75, 89). Tandem duplication of the control region is comparatively common in insects, being found in individual species in multiple orders. Nontandem duplications, i.e., those separated by protein-coding genes, are much rarer, being found in metastriate ticks (133), some parrots (35), and thrips (127). Two separate control regions are retained in conserved genomic positions across Thripinae, and three are conserved in *Frankliniella* spp. (89). At present, it is unknown if all of these putative control regions recorded in thrips are functional; however, their retention across approximately 60 million years in Thripinae suggests functionality (89). Duplicated control regions likely do not increase mt gene rearrangement rates, as was once proposed (see 127); however, their impact on nucleotide substitution rates or compositional biases has yet to be thoroughly tested. These factors differ significantly between thrips families and are correlated with gene rearrangement rate (89); however, control region duplication is an untested, potentially confounding factor in that analysis. Again, the multiple independent origins of duplicated control regions and their retention through deep time provide a powerful means

to test how genome structure constrains nucleotide evolution and thus can refine methods that assume neutral mutation dynamics (e.g., DNA barcoding, dating analysis).

FUTURE DIRECTIONS IN INSECT MITOCHONDRIAL GENOMES

Finally, the increasing body of work examining animal mt genomes from a functional perspective has yet to be thoroughly applied to insects. Mitonuclear incompatibility (50) describes the phenomena whereby coadaptation between nuclear and mt-encoded genes is necessary for efficient respiratory function and is a potential mechanism for speciation through reproductive isolation; i.e., hybrids have less fit mitonuclear combinations (117). Although this phenomenon is best studied in birds (49), there are several well-studied insect examples of mitonuclear incompatibility, including leaf beetles (94) and fruit flies (63). Evolutionary rates between mt- and nuclear-encoded mitochondrial genes are strongly correlated in insects (139), suggesting compensatory mutations between the two, a prerequisite for mitonuclear incompatibility. While mitonuclear incompatibility has been proposed as explaining the DNA barcode gap observed between many good biological species (49), the barcode gap is often absent in insects (81). A range of genomic phenomena, including those discussed in this review (e.g., gene rearrangements, genome fragmentation, control region duplication, recombination, selection), as well as others not covered [e.g., reproductive symbionts (21)], can affect mt nucleotide divergence rates during allopatric speciation, undermining predictable mitonuclear incompatibility formation (see 15). Presently, it is unknown how widespread and intense mitonuclear incompatibility is within insects and what confounding effects other aspects of mt genomic biology may have; resolution of these questions would improve the reliability of DNA-based taxonomy in insects.

A second functional perspective on mt genomic evolution has been examining selective adaption to extreme environments (e.g., 14). Because the mt genome encodes subunits of the OXPHOS pathway, both environmental oxygen deprivation and temperature stress could potentially put selective pressure on mt genes. Purifying (negative) selection is nearly ubiquitous for insect mt genes, typically demonstrated by *dN/dS* ratios significantly <1 for interspecies comparisons. Adaptive (positive) selection has been far less frequently demonstrated, with examples including adaptation to high altitudes [in wasps (58), butterflies (144), and grasshoppers (69)], diet [in ladybugs (143)], and flight [in grasshoppers (69)]. Methods used in these studies are again mostly restricted to mt *dN/dS* ratios calculated at interspecies scales. More systematic tests of environmental adaption, including the use of phylogenetically independent contrasts, tests for convergent adaptation, sister-clade sampling around putative environmental transitions (e.g., high versus low altitude), population-level sequencing, and parallel studies of nuclear genomic adaption (e.g., 97), would contribute to a more rigorous understanding of the balance between purifying versus adaptive evolution in insect mt genomes.

SUMMARY POINTS

1. Next-generation sequencing has facilitated a rapid increase in insect mitochondrial (mt) genomes. Mt genomes can be assembled as bycatch from most nuclear phylogenomic sequencing methods.
2. Available mt genomes broadly track species-level diversity within insect orders. Coleoptera, Hymenoptera, Ephemeroptera, Odonata, and Siphonaptera are, however, comparatively undersampled relative to their diversity.

3. Mt and nuclear phylogenomic reconstruction of ordinal relationships are congruent for a majority of orders, but combined analyses are sorely needed.
4. Expanded sampling supports the hypothesized relationship between haplodiploidy and increased mt genome rearrangement rates (19) for multiple orders but does not hold in Coleoptera. The mechanism of this connection is still unstudied.
5. Insect systems provide models for rare mt genomic phenomena, such as fragmentation and control region duplication, that improve our general understanding of genome function.
6. Mitonuclear incompatibility and mt adaptive evolution are emerging areas of research in insects with significant potential applications across entomology.

DISCLOSURE STATEMENT

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