A model species for agricultural pest genomics: the genome of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Abstract

The Colorado potato beetle is one of the most challenging agricultural pests to manage. It has shown a spectacular ability to adapt to a variety of solanaceaeous plants and variable climates during its global invasion, and, notably, to rapidly evolve insecticide resistance. To examine evidence of rapid evolutionary change, and to understand the genetic basis of herbivory and insecticide resistance, we tested for structural and functional genomic changes relative to other arthropod species using genome sequencing, transcriptomics, and community annotation. Two factors that might facilitate rapid evolutionary change include transposable elements, which comprise at least 17% of the genome and are rapidly evolving compared to other Coleoptera, and high levels of nucleotide diversity in rapidly growing pest populations. Adaptations to plant feeding are evident in gene expansions and differential expression of digestive enzymes in gut tissues, as well as expansions of gustatory receptors for bitter tasting. Surprisingly, the suite of genes involved in insecticide resistance is similar to other beetles. Finally, duplications in the RNAi pathway might explain why *Leptinotarsa decemlineata* has high sensitivity to dsRNA. The *L. decemlineata* genome provides opportunities to investigate a broad range of phenotypes and to develop sustainable methods to control this widely successful pest.

Keywords: insects; evolution; pesticide resistance; herbivory; plant-insect interactions; pest management; whole-genome sequence

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae), is widely considered one of the world's most successful globally-invasive insect herbivores, with costs of ongoing management reaching tens of millions of dollars annually [1] and projected costs if unmanaged reaching billions of dollars [2]. This beetle was first identified as a pest in 1859 in the Midwestern United States, after it expanded from its native host plant, *Solanum rostratum* (Solanaceae), onto potato (*S. tuberosum*) [3]. As testimony to the difficulty in controlling *L. decemlineata*, the species has the dubious honor of starting the pesticide industry, when Paris Green (copper (II) acetoarsenite) was first applied to control it in the United States in 1864 [4]. *Leptinotarsa decemlineata* is now widely recognized for its ability to rapidly evolve resistance to insecticides, as well as a wide range of abiotic and biotic stresses [5], and for its global expansion across 16 million km^2 to cover the entire Northern Hemisphere within the 20th century [6]. Over the course of 150 years of research, *L. decemlineata* has been the subject in more than 9,700 publications (according to the Web of Science™ Core Collection of databases) ranging from molecular to organismal biology from the fields of agriculture, entomology, molecular biology, ecology, and evolution.

In order to be successful, *L. decemlineata* evolved to exploit novel host plants, to inhabit colder climates at higher latitudes [7–9], and to cope with a wide range of novel environmental conditions in agricultural landscapes [10,11]. Genetic data suggest the potato-feeding pest lineage directly descended from populations that feed on *S. rostratum* in the U.S. Great Plains [12]. This beetle subsequently expanded its range northwards, shifting its life history strategies to exploit even colder climates [7,8,13], and steadily colonized potato crops despite substantial geographical barriers [14]. *Leptinotarsa decemlineata* is an excellent model for understanding

pest evolution in agroecosystems because, despite its global spread, individuals disperse over short distances and populations often exhibit strong genetic differentiation [15–17], providing an opportunity to track the spread of populations and the emergence of novel phenotypes. The development of genomic resources in *L. decemlineata* will provide an unparalleled opportunity to investigate the molecular basis of traits such as climate adaptation, herbivory, host expansion, and chemical detoxification. Perhaps most significantly, understanding its ability to evolve rapidly would be a major step towards developing sustainable methods to control this widely successful pest in agricultural settings.

Given that climate is thought to be the major factor in structuring the range limits of species [18], the latitudinal expansion of *L. decemlineata,* spanning more than 40° latitude from Mexico to northern potato-producing countries such as Canada and Russia [6], warrants further investigation. Harsh winter climates are thought to present a major barrier for insect range expansions, especially near the limits of a species' range [7,19]. To successfully overwinter in temperate climates, beetles need to build up body mass, develop greater amounts of lipid storage, have a low resting metabolism, and respond to photoperiodic keys by initiating diapause [20,21]. Although the beetle has been in Europe for less than 100 years, local populations have demonstrating remarkably rapid evolution in life history traits linked to growth, diapause and metabolism [8,13,20]. Understanding the genetic basis of these traits, particularly the role of specific genes associated with metabolism, fatty acid synthesis, and diapause induction, could provide important information about the mechanism of climate adaptation.

Although *Leptinotarsa decemlineata* has long-served as a model for the study of host expansion and herbivory due to its rapid ability to host switch [17,22], a major outstanding question is what genes and biological pathways are associated with herbivory in this species?

While >35,000 species of Chrysomelidae are well-known herbivores, most species feed on one or a few host species within the same plant family [23]. Within *Leptinotarsa*, the majority of species feed on plants within Solanaceae and Asteraceae, while *L. decemlineata* feeds exclusively on solanaceous species [24]. It has achieved the broadest host range amongst its congeners (including, but not limited to: buffalobur (*S. rostratum*), potato (*S. tuberosum*), eggplant (*S. melongena*), silverleaf nightshade (*S. elaeagnifolium*), horsenettle (*S. carolinense*), bittersweet nightshade (*S. dulcamara*), tomato *(S. lycopersicum*), and tobacco (*Nicotiana tabacum*)) [17,22,25], and exhibits geographical variation in the use of locally abundant *Solanum* species [26].

Another major question is what are the genes that underlie the beetle's remarkable capacity to detoxify plant secondary compounds and are these the same biological pathways used to detoxify insecticidal compounds [27]? Solanaceous plants are considered highly toxic to a wide range of insect herbivore species [28], because they contain steroidal alkaloids and glycoalkaloids, nitrogen-containing compounds that are toxic to a wide range of organisms, including bacteria, fungi, humans, and insects [29], as well as glandular trichomes that contain additional toxic compounds [30]. In response to beetle feeding, potato plants upregulate pathways associated with terpenoid, alkaloid, and phenylpropanoid biosynthesis, as well as a range of protease inhibitors [31]. A complex of digestive cysteine proteases is known to underlie *L. decemlineata*'s ability to respond to potato-induced defenses [32,33]. There is evidence that larvae excrete [34] and perhaps even sequester toxic plant-based compounds in the hemolymph [35,36]. Physiological mechanisms involved in detoxifying plant compounds, as well as other xenobiotics, have been proposed to underlie pesticide resistance [27]. To date, while cornerstone of *L. decemlineata* management has been the use of insecticides, the beetle has evolved

resistance to over 50 compounds and all of the major classes of insecticides. Some of these chemicals have even failed to control *L. decemlineata* within the first year of release [10], and notably, regional populations of *L. decemlineata* have demonstrated the ability to independently evolve resistance to pesticides and to do so at different rates [37]. Previous studies have identified target site mutations in resistance phenotypes and a wide range of genes involved in metabolic detoxification, including carboxylesterase genes, cytochrome P450s, and glutathione S-transferase genes [38–42].

To examine evidence of rapid evolutionary change underlying *L. decemlineata*'s extraordinary success utilizing novel host plants, climates, and detoxifying insecticides, we evaluated structural and functional genomic changes relative to other beetle species, using whole-genome sequencing, transcriptome sequencing, and a large community-driven biocuration effort to improve predicted gene annotations. We compared the size of gene families associated with particular traits against existing available genomes from related species, particularly those sequenced by the i5k project (http://i5k.github.io), an initiative to sequence 5,000 species of Arthropods. While efforts have been made to understand the genetic basis of phenotypes in *L. decemlineata* (for example, pesticide resistance) [32,43,44], previous work has been limited to candidate gene approaches rather than comparative genomics. Genomic data can not only illuminate the genetic architecture of a number of phenotypic traits that enable *L. decemlineata* to continue to be an agricultural pest, but can also be used to identify new gene targets for control measures. For example, recent efforts have been made to develop RNAi-based pesticides targeting critical metabolic pathways in *L. decemlineata* [41,45,46]. With the extensive wealth of biological knowledge and a newly-released genome, this beetle is well-positioned to be a model system for agricultural pest genomics and the study of rapid evolution.

Results and Discussion

Genome Assembly, Annotation and Assessment

A single female *L. decemlineata* from Long Island, NY, USA, a population known to be resistant to a wide range of insecticides [47,48], was sequenced to a depth of ~140x coverage and assembled with ALLPATHS [49] followed by assembly improvement with ATLAS (https://www.hgsc.bcm.edu/software/). The average coleopteran genome size is 760 Mb (ranging from 160-5,020 Mb [50]), while most of the beetle genome assemblies have been smaller (mean assembly size 286 Mb, range 160-710 Mb) [51-55]. The draft genome assembly of *L. decemlineata* is 1.17 Gb and consists of 24,393 scaffolds, with a N50 of 414 kb and a contig N50 of 4.9 kb. This assembly is more than twice the estimated genome size of 460 Mb [56], with the presence of gaps comprising 492 Mb, or 42%, of the assembly. As this size might be driven by underlying heterozygosity, we also performed scaffolding with REDUNDANS [57], which reduced the assembly size to 642 Mb, with gaps reduced to 1.3% of the assembly. However, the REDUNDANS assembly increased the contig N50 to 47.4 kb, the number of scaffolds increased to 90,205 and the N50 declined to 139 kb. By counting unique 19 bp kmers and adjusting for ploidy, we estimate the genome size as 816.9 Mb. Using just the small-insert (180 bp) 100 bp PE reads, average coverage was 24X for the ALLPATHS assembly and 26X for the REDUNDANS assembly. For all downstream analyses, the ALLPATHS assembly was used due to its increased scaffold length and reduced number of scaffolds.

The number of genes in the *L. decemlineata* genome predicted based on automated annotation using MAKER was 24,671 gene transcripts, with 93,782 predicted exons, which surpasses the 13,526-22,253 gene models reported in other beetle genome projects (**Figure 1**)

[51-55]. This may be in part due to fragmentation of the genome, which is known to inflate gene number estimates [58]. To improve our gene models, we manually annotated genes using expert opinion and additional mRNA resources (see **Supplementary File** for more details). A total of 1,364 genes were manually curated and merged with the unedited MAKER annotations to produce an official gene set (OGS v1.0) of 24,850 transcripts, comprised of 94,859 exons. A total of 12 models were curated as pseudogenes. A total of 1,237 putative transcription factors (TFs) were identified in the *L. decemlineata* predicted proteome (**Figure 2**). The predicted number of TFs is similar to some beetles, such as *Anoplophora glabripennis* (1,397) and *Hypothenemus hampei* (1,148), but substantially greater than others, such as *Tribolium castaneum* (788), *Nicrophorus vespilloides* (744), and *Dendroctonus ponderosae* (683) [51-55].

We assessed the completeness of both the ALLPATHS and REDUNDANS assemblies, and the OGS separately, using benchmarking sets of universal single-copy orthologs (BUSCOs) based on 35 holometabolous insect genomes [59], as well as manually assessing the completeness and co-localization of the homeodomain transcription factor gene clusters in the ALLPATHS assembly. Using the reference set of 2,442 BUSCOs, the ALLPATHS genome assembly, REDUNDANS genome assembly, and OGS were 93.0%, 91.9%, and 71.8% complete, respectively. We found an additional 4.1%, 5.4%, and 17.9% of the BUSCOs present but fragmented in each dataset, respectively. For the highly conserved Hox and Iroquois Complex (Iro-C) clusters, we located and annotated complete gene models in the ALLPATHS genome assembly for all 12 expected orthologs, but these were split across six different scaffolds (**Supplementary Figure 1S** and **Table 1S**). All linked Hox genes occurred in the expected order and with the expected, shared transcriptional orientation, suggesting that the current draft assembly was correct but incomplete (see also **Supplementary Figures 2S** and **3S**). Assuming

direct concatenation of scaffolds, the Hox cluster would span a region of 3.7 Mb, similar to the estimated 3.5 Mb Hox cluster of *A. glabripennis* [54]. While otherwise highly conserved with *A. glabripennis*, we found a tandem duplication for *Hox3/zen* and an Antennapedia-class (ANTP-class) homeobox gene with no clear ortholog in other arthropods. We also assessed the ALLPATHS genome assembly for evidence of contamination using a Blobplot (**Supplementary Figure 4S**), which identified a small proportion of the reads (5.9%) as putative contaminants (the largest single taxonomic group represented, 2.6%, was assigned to Annelida).

Gene Annotation, Gene Family Evolution and Differential Expression

We estimated a phylogeny among six coleopteran genomes (*A. glabripennis, Agrilus planipennis, D. ponderosae*, *L. decemlineata*, *Onthophagus taurus*, and *T. castaneum*; unpublished genomes available at http://i5k.github.io/) [51,52,54] using a conserved set of single copy orthologs and compared the official gene set of each species to understand how gene families evolved the branch representing Chysomelidae. *Leptinotarsa decemlineata* and *A. glabripennis* (Cerambycidae) are sister taxa (**Figure 1**), as expected for members of the same superfamily Chrysomeloidea. We found 166 rapidly evolving gene families along the *L. decemlineata* lineage (1.4% of 11,598), 142 of which are rapid expansions and the remaining 24 rapid contractions **(Table 1)**. Among all branches of our coleopteran phylogeny, *L. decemlineata* has the highest average expansion rate (0.203 genes per million years), the highest number of genes gained, and the greatest number of rapidly evolving gene families.

Examination of the functional classification of rapidly evolving families in *L. decemlineata* (**Supplementary Tables 2S** and **3S**) indicates that a subset of families are clearly associated with herbivory. The peptidases, comprising several gene families that play a major

role in plant digestion [32,60], displayed a significant expansion in genes (OrthoDB family Id: EOG8JDKNM, EOG8GTNV8, EOG8DRCSN, EOG8GTNT0, EOG8K0T47, EOG88973C, EOG854CDR, EOG8Z91BB, EOG80306V, EOG8CZF0X, EOG80P6ND, ,EOG8BCH22, EOG8ZKN28, EOG8F4VSG, EOG80306V, EOG8BCH22, EOG8ZKN28, EOG8F4VSG). While olfactory receptor gene families have rapidly contracted (EOG8Q5C4Z, EOG8RZ1DX), subfamilies of odorant binding proteins and gustatory receptors have grown (see Sensory Ecology section below). The expansion of gustatory receptor subfamilies are associated with bitter receptors, likely reflecting host plant detection of nightshades (Solanaceae). Some gene families associated with plant detoxification and insecticide resistance have rapidly expanded (Glutathione S-transferases: EOG85TG3K, EOG85F05D, EOG8BCH22; UDP-glycosyltransferase: EOG8BCH22; cuticle proteins: EOG8QJV4S, EOG8DNHJQ; ABC transporters: EOG83N9TJ), whereas others have contracted (Cytochrome P450s: EOG83N9TJ). Finally, gene families associated with immune defense (fibrinogen: EOG8DNHJQ; Immunoglobulin: EOG8Q87B6, EOG8S7N55, EOG854CDX, EOG8KSRZK, EOG8CNT5M) exhibit expansions that may be linked to defense against pathogens and parasitoids that commonly attack exposed herbivores [61]. A substantial proportion of the rapidly evolving gene families include proteins with transposable element domains (25.3%), while other important functional groups include DNA and protein binding (including many transcription factors), nuclease activity, protein processing, and cellular transport.

Diversification of transcription factor (TF) families potentially signals greater complexity of gene regulation, including enhanced cell specificity and refined spatiotemporal signaling [62]. Notably, several TF families are substantially expanded in *L. decemlineata*, including HTH_psq (194 genes vs. a mean of only 24 across the insects shown in **Figure 2**), MADF (152 vs. 54), and

271 THAP (65 vs. 41). Two of these TF families, HTH_psq and THAP, are DNA binding domains in
272 transposons. Of the 1,237 *L. decemlineata* TFs, we could infer DNA binding motifs for 189
273 (15%) (**Supplementary Table 4S**), mostly based on DNA binding specificity data from
274 *Drosophila melanogaster* (124 TFs), but also from species as distant as human (45 TFs) and
275 mouse (11 TFs). Motifs were inferred for a substantial proportion of the TFs in the largest TF
276 families, including Homeodomain (59 of 90, 66%), bHLH (34 of 46, 74%), and Forkhead box
277 (14 of 16, 88%). We could only infer a small number of C2H2 zinc finger motifs (21 of 439,
278 ~5%), which is expected as these sequences evolve quickly by shuffling zinc finger arrays,
279 resulting in largely dissimilar DNA-binding domains across metazoans [63]. Collectively, the
280 almost 200 inferred DNA binding motifs for *L. decemlineata* TFs provide a unique resource to
281 begin unraveling gene regulatory networks in this organism.

282 To identify genes active in mid-gut tissues, life-stages, and sex differences, we examined
283 differential transcript expression levels using RNA sequencing data. Comparison of significantly
284 differentially expressed genes with >100-fold change, after Bonferroni correction, indicated
285 higher expression of digestive enzymes (proteases, peptidases, dehydrogenases and transporters)
286 in mid-gut versus whole larval tissues, while cuticular proteins were largely expressed at lower
287 levels (**Figure 3A, Supplementary Table 5S**). Comparison of an adult male and female showed
288 higher expression of testes and sperm related genes in males, while genes involved in egg
289 production and sterol biosynthesis are more highly expressed in females (**Figure 3B,**
290 **Supplementary Table 6S**). Comparisons of larvae to both an adult male (**Figure 3C,**
291 **Supplementary Table 7S**) and an adult female (**Figure 3D, Supplementary Table 8S**) showed
292 higher expression of larval-specific cuticle proteins, and lower expression of odorant binding and
293 chemosensory proteins. The adults, both drawn from a pesticide resistant population, showed

294 higher constitutive expression of cytochrome p450 genes compared to the larval population,
295 which is consistent with the results from previous studies of neonicotinoid resistance in this
296 population [48].

297 **Transposable Elements**

298 Transposable elements (TEs) are ubiquitous mobile elements within most eukaryotic
299 genomes and play critical roles in both genome architecture and the generation of genetic
300 variation [64]. Through insertional mutagenesis and recombination, TEs are a major contributor
301 to the generation of novel mutations (50-80% of all mutation events within the genome of *D.*
302 *melanogaster*) [65-67], and are increasingly thought to generate much of the genetic diversity
303 that contributes to rapid evolution [68–70]. In addition to finding that genes with TE domains
304 comprise 25% of the rapidly evolving gene families within *L. decemlineata*, we found that at
305 least 17% of the genome consists of TEs (**Supplementary Table 9S**). This is substantially
306 greater than the 6% found in *T. castaneum* [51], but less than some Lepidoptera (35% in *Bombyx*
307 *mori* and 25% in *Heliconius melpomene*) [71,72]. LINEs were the largest TE class, comprising
308 ~10% of the genome, while SINEs were not detected. Curation of the TE models with intact
309 protein domains resulted in 334 current models of potentially active TEs, meaning that these TEs
310 are capable of transposition and excision. Within the group of active TEs, we found 191 LINEs,
311 99 DNA elements, 38 LTRs, and 5 Helitrons. Given that TEs have been associated with the
312 ability of species to rapidly adapt to novel selection pressures [73–75], particularly via alterations
313 of gene expression patterns in neighboring genomic regions, we scanned gene rich regions (1 kb
314 neighborhood size) for active TE elements. Genes with active neighboring TEs have functions
315 that include transport, protein digestion, diapause, and metabolic detoxification (**Supplementary**
316 **Table 10S**). Because TE elements have been implicated in conferring insecticide resistance in

317 other insects [76], future work should investigate the role of these TE insertions on rapid
318 evolutionary changes within pest populations of *L. decemlineata*.
319
320 **Population Genetic Variation and Invasion History**
321 To understand the propensity for *L. decemlineata* pest populations to rapidly evolve
322 across a range of environmental conditions, we examined geographical patterns of genomic
323 variability and the evolutionary history of *L. decemlineata* (to see the genomic analysis of
324 environmental adaptation, refer to the Diapause and Environmental Stress section of
325 **Supplementary File**). High levels of standing variation are one mechanism for rapid
326 evolutionary change [77]. We identified 1.34 million biallelic single nucleotide polymorphisms
327 (SNPs) from pooled RNAseq datasets, or roughly 1 variable site for every 22 base pairs of
328 coding DNA. This rate of polymorphism is exceptionally high when compared to vertebrates
329 (e.g. ~1 per kb in humans, or ~1 per 500 bp in chickens) [78,79], and is 8-fold higher than other
330 beetles (1 in 168 for *D. ponderosae* and 1 in 176 bp for *O. taurus*) [52,80] and 2 to 5-fold higher
331 than some dipterans (1 in 54 bp for *D. melanogaster* and 1 in 125 bp for *Anopheles gambiae*)
332 [78,81]. It is likely that these values simply scale with effective population size, although the
333 dipterans, with the largest known population sizes, have reduced variation due to widespread
334 selective sweeps and genetic bottlenecks [82].
335 Evolutionary relationships and the amount of genetic drift among Midwestern USA,
336 Northeastern USA, and European *L. decemlineata* populations were estimated based on genome-
337 wide allele frequency differences using a population graph. A substantial amount of local genetic
338 structure and high genetic drift is evident among all populations, although both the reference lab
339 strain from New Jersey and European populations appear to have undergone more substantial

drift, suggestive of strong inbreeding (**Figure 4**). Population genetic divergence (F_{ST}) values (**Supplementary Table 11S**) range from 0.035 (among Wisconsin populations) to 0.182 (Wisconsin compared to Europe). The allele frequency spectrum was calculated for populations in Wisconsin, Michigan, and Europe to estimate the population genetic parameter θ, or the product of the mutation rate and the ancestral effective population size, and the ratio of contemporary to ancestral population size in models that allowed for single or multiple episodes of population size change. Estimates of ancestral θ are much higher for Wisconsin (θ = 12595) and Michigan (θ = 93956) than Europe (θ = 3.07; **Supplementary Table 12S**), providing support for a single introduction into Europe following a large genetic bottleneck [15]. In all three populations, a model of population size growth is supported, in agreement with historical accounts of the beetles expanding from the Great Plains into the Midwestern U.S. and Europe [3,15], but the dynamics of each population appear independent, with the population from Michigan apparently undergoing a very recent decline in contemporary population size (the ratio of contemporary to ancient population size is 0.066, compared to 3.3 and 2.1 in Wisconsin and Europe, respectively).

Sensory Ecology and Host Plant Detection

To interact with their environment, insects have evolved neurosensory organs to detect environmental signals, including tactile, auditory, chemical and visual cues [83]. We examined neural receptors, olfactory genes, and light sensory (opsin) genes to understand the sensory ecology and host-plant specializations of *L. decemlineata*.

We found high sequence similarity in the neuroreceptors of *L. decemlineata* compared to other Coleoptera. The transient receptor potential (TRP) channels are permeable transmembrane proteins that respond to temperature, touch, pain, osmolarity, pheromones, taste, hearing, smell

and visual cues of insects [84,85]. In most insect genomes, there are typically 13-14 TRP genes located in insect stretch receptor cells and several are targeted by commercial insecticides [86]. We found 12 TRP genes present in the *L. decemlineata* genome, including the two TRPs (Nanchung and Inactive) that are targeted by commercial insecticides, representing a complete set of one-to-one orthologs with *T. castaneum.* Similarly, the 20 known amine neurotransmitter receptors in *T. castaneum* are present as one-to-one orthologs in *L. decemlineata* [87,88]. Amine receptors are G-protein-coupled receptors that interact with biogenic amines, such as octopamine, dopamine and serotonin. These neuroactive substances regulate behavioral and physiological traits in animals by acting as neurotransmitters, neuromodulators and neurohormones in the central and peripheral nervous systems [89].

The majority of phytophagous insects are restricted to feeding on several plant species within a genus, or at least restricted to a particular plant family [90]. Thus, to find their host plants within heterogeneous landscapes, insect herbivores detect volatile organic compounds through olfaction, which utilizes several families of chemosensory gene families, such as the odorant binding proteins (OBPs), odorant receptors (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) [91]. OBPs directly bind with volatile organic compounds emitted from host plants and transport the ligands across the sensillar lymph to activate the membrane-bound ORs in the dendrites of the olfactory sensory neurons [92]. The ORs and GRs are 7-transmembrane proteins related within a superfamily [93] of ligand-gated ion channels [94]. The ionotropic receptors are related to ionotropic glutamate receptors and function in both smell and taste [95]. These four gene families are commonly large in insect genomes, consisting of tens to hundreds of members.

We compared the number of genes found in *L. decemlineata* in the four chemosensory gene families to *T. castaneum* and *A. glabripennis* (**Table 2**, as well as **Supplementary Tables 13S-16S** for details). While the OBP family is slightly enlarged, the three receptor families are considerably smaller in *L. decemlineata* than in either *A. glabripennis* or *T. castaneum*, consistent with the specialization of this beetle on one genus of plants. However, each beetle species exhibits species-specific gene subfamily expansions (**Supplementary Figures 5S-8S**); in particular, some lineages of GRs related to bitter taste are expanded in *L. decemlineata* relative to *A. glabripennis* and other beetles. Among the OBPs, we identified a major *L. decemlineata*-specific expansion of proteins belonging to the Minus-C class (OBPs that have lost two of their six conserved cysteine residues) that appear unrelated to the 'traditional' Minus-C subfamily in Coleoptera, indicating that coleopteran OBPs have lost cysteines on at least two occasions.

To understand the visual acuity of *L. decemlineata*, we examined the G-protein-coupled transmembrane receptor opsin gene family. We found five opsins, three of which are members of rhabdomeric opsin (R-opsin) subfamilies expressed in the retina of insects [51,96]. Specifically, the *L. decemlineata* genome contains one member of the long wavelength-sensitive R-opsin and two short wavelength UV-sensitive R-opsins. The latter were found to be closely linked in a range of less than 20,000 bp, suggestive of recent tandem gene duplication. Overall, the recovered repertoire of retinally-expressed opsins in *L. decemlineata* [97] is consistent with the beetle's attraction to yellow light and to the yellow flowers of its ancestral host plant, *S. rostratum* [98,99], and is consistent with the beetle's sensitivity in the UV- and LW-range [100]. In addition, we found a member of the Rh7 R-opsin subfamily, which is broadly conserved in insects including other beetle species (*A. glabripennis*), although it is missing from *T.*

castaneum. Finally, *L. decemlineata* has a single ortholog of the c-opsin subfamily shared with *T. castaneum*, which is absent in *A. glabripennis* and has an unclear role in photoreception [101].

Host Plant Utilization

Protein digestion

Insect herbivores are fundamentally limited by nitrogen availability [102], and thus need to efficiently break down plant proteins in order to survive and develop on host plants [103]. *Leptinotarsa decemlineata* has serine and cysteine digestive peptidases (coined "intestains") [32,104,105], as well as aspartic and metallo peptidases [33], for protein digestion. For the vast majority of plant-eating beetles (the infraorder Cucujiformia, which includes Chrysomelidae), cysteine peptidases contribute most strongly to proteolytic activity in the gut [105,106]. In response to herbivory, plants produce a wide range of proteinase inhibitors to prevent insect herbivores from digesting plant proteins [103,105,107]. Coleopteran peptidases are differentially susceptible to plant peptidase inhibitors, and our annotation results suggest that gene duplication and selection for inhibitor insensitive genotypes may have contributed to the success of leaf-feeding beetles (Chrysomelidae) on different plants. We found that gene expansion of cysteine cathepsins from the C1 family in *L. decemlineata* correlates with the acquisition of greater digestive function by this group of peptidases, which is supported by gene expression activity of these genes in mid-gut tissue (**Figure 3A, Figure 5**). The gene expansion may be explained by an evolutionary arms race between insects and plants that favors insects with a variety of digestive peptidases in order to resist plant peptidase inhibitors [108,109] and allows for functional specialization [110].

Cysteine peptidases of the C1 family were represented by more than 50 genes separated into four groups with different structure and functional characteristics (**Supplementary Table 18S**): cathepsin L subfamily, cathepsin B subfamily, TINAL-like genes, and cysteine peptidase inhibitor domains (CPIDs). Cathepsin L subfamily cysteine peptidases are endopeptidases [111] that can be distinguished by the cathepsin propeptide inhibitor domain I29 (pfam08246) [112,113]. Within the cathepsin L subfamily, we found sequences that were similar to classical cathepsin L, cathepsin F, and cathepsin O. However, there were 28 additional predicted peptidases of this subfamily that could not be assigned to any of the "classical" cathepsin types, and most of these were grouped into two gene expansions (uL1 and uL2) according to their phylogenetic and structural characteristics. Cathepsin B subfamily cysteine peptidases are distinguished by the specific peptidase family C1 propeptide domain (pfam08127). Within the cathepsin B subfamily, there was one gene corresponding to typical cathepsin B peptidases and 14 cathepsin B-like genes. According to the structure of the occluding loop, only the typical cathepsin B may have typical endo- and exopeptidase activities, while a large proportion of cathepsin B-like peptidases presumably possesses only endopeptidase activity due to the absence of a His-His active subsite in the occluding loop, which is responsible for exopeptidase activity [111]. Only one gene corresponding to a TINAL-like-protein was present, which has a domain similar to cathepsin B in the C-terminus, but lacks peptidase activity due to the replacement of the active site Cys residue with Ser [114]. Cysteine peptidase inhibitor domain (CPID) genes encode the I29 domain of cysteine peptidases without the mature peptidase domain. Within the CPID group, there were seven short inhibitor genes that lack the enzymatic portion of the protein. A similar trend of "stand-alone inhibitors" has been observed in other insects, such as *B. mori* [115]. These CPID genes may be involved in the regulation of cysteine peptidases. We note

453 that we found multiple fragments of cysteine peptidase genes, suggesting that the current list of
454 *L. decemlineata* genes may be incomplete. Comparison of these findings with previous data on
455 *L. decemlineata* cysteine peptidases [116] demonstrates that intestains correspond to several
456 peptidase genes from the uL1 and uL2 groups (**Supplementary Table 18S**). These data, as well
457 as literature for Tenebrionidae beetles [117], suggest that intensive gene expansion is typical for
458 peptidases that are involved in digestion.

459 We also found a high number of digestion-related serine peptidase genes in the *L.*
460 *decemlineata* genome (**Supplementary Table 19S**), but they contribute only a small proportion
461 of the beetle's total gut proteolytic activity [32]. Of the 31 identified serine peptidase genes and
462 fragments, we annotated 16 as trypsin-like peptidases and 15 as chymotrypsin-like peptidases.
463 For four chymotrypsin-like and one trypsin-like peptidase, we identified only short fragments.
464 All complete (and near-complete) sequences have distinctive S1A peptidase subfamily motifs, a
465 conserved catalytic triad, conserved sequence residues such as the "CWC" sequence and
466 cysteines that form disulfide bonds in the chymotrypsin protease fold. The number of serine
467 peptidases was higher than expected based upon the number of previously identified EST clones
468 [32], but lower than the number of chymotrypsin and trypsin genes in the *T. castaneum* genome.
469
470 *Carbohydrate digestion*

471 Carbohydrates are the other category of essential nutrients for *L. decemlineata*. The
472 enzymes that assemble and degrade oligo- and polysaccharides, collectively termed
473 Carbohydrate active enzymes (CAZy), are categorized into five major classes: glycoside
474 hydrolases (GH), polysaccharide lyases, carbohydrate esterases (CE), glycosyltransferases (GT)
475 and various auxiliary oxidative enzymes [118]. Due to the many different roles of carbohydrates,

476 the CAZy family profile of an organism can provide insight into "glycobiological potential" and,
477 in particular, mechanisms of carbon acquisition [119]. We identified 182 GHs assigned to 25
478 families, 181 GTs assigned to 41 families, and two CEs assigned to two families in *L.*
479 *decemlineata*; additionally, 99 carbohydrate-binding modules (which are non-catalytic modules
480 associated with the above enzyme classes) were present and assigned to 9 families
481 (**Supplementary Table 20S**; the list of CAZy genes is presented in **Supplementary Table 21S**).
482 We found that *L. decemlineata* has three families of genes associated with plant cell wall
483 carbohydrate digestion (GH28, GH45 and GH48) that commonly contain enzymes that target
484 pectin (GH28) and cellulose (GH45 and GH48), the major structural components of leaves [120].
485 We found evidence of massive gene duplications in the GH28 family (14 genes) and GH45
486 family (11 genes, plus one additional splicing variant), whereas GH48 is represented by only
487 three genes in the genome [121]. Overall, the genome of *L. decemlineata* shows a CAZy profile
488 adapted to metabolize pectin and cellulose contained in leaf cell walls. The absence of specific
489 members of the families GH43 (α-L-arabinofuranosidases) and GH78 (α-L-rhamnosidases)
490 suggests that *L. decemlineata* can break down homogalacturonan, but not substituted
491 galacturonans such as rhamnogalacturonan I or II [120]. The acquisition of these plant cell wall
492 degrading enzymes has been linked to horizontal transfer in the leaf beetles and other
493 phytophagous beetles [120], with strong phylogenetic evidence supporting the transfer of GH28
494 genes from a fungal donor (Pezizomycotina) in *L. decemlineata*, as well as in the beetles *D.*
495 *ponderosae* and *Hypothenemus hampei*, but a novel fungal donor in the more closely related
496 cerambycid beetle *A. glabripennis* [54] and a bacterial donor in the weevil *Callosobruchus*
497 *maculatus* [120].
498

Insecticide Resistance

To understand the functional genomic properties of insecticide resistance, we examined genes important to neuromuscular target site sensitivity, tissue penetration, and prominent gene families involved in Phase I, II, and III metabolic detoxification of xenobiotics [122]. These include the cation-gated nicotinic acetylcholine receptors (nAChRs), the γ-amino butyric acid (GABA)-gated anion channels and the histamine-gated chloride channels (HisCls), cuticular proteins, cytochrome P450 monooxygenases (CYPs), and the Glutathione S-transferases (GSTs).

Many of the major classes of insecticides (organochlorides, organophosphates, carbamates, pyrethroids, neonicotinoids and ryanoids) disrupt the nervous system (particularly ion channel signaling), causing paralysis and death [123]. Resistance to insecticides can come from point mutations that reduce the affinity of insecticidal toxins to ligand-gated ion superfamily genes [124]. The cys-loop ligand-gated ion channel gene superfamily is comprised of receptors involved in mediating synaptic ion flow during neurotransmission [125]. A total of 22 cys-loop ligand-gated ion channels were identified in the *L. decemlineata* genome in numbers similar to those observed in other insects [126], including 12 nAChRs, three GABA receptors, and two HisCls (**Supplementary Figure 9S**). The GABA-gated chloride channel homolog of the *Resistance to dieldrin* (*Rdl*) gene of *D. melanogaster* was examined due to its role in resistance to dieldrin and other cyclodienes in Diptera [127]. The coding sequence is organized into 10 exons (compared to nine in *D. melanogaster*) on a single scaffold, with duplications of the third and sixth exon (**Supplementary Figure 10S**). Alternative splicing of these two exons encodes for four different polypeptides in *D. melanogaster* [128,129], and as the splice junctions are present in *L. decemlineata*, we expect the same diversity of *Rdl*. The point mutations in the

521 transmembrane regions TM2 and TM3 of *Rdl* are known to cause insecticide resistance in
522 Diptera [124,127], but were not observed in *L. decemlineata*.

523 Cuticle genes have been implicated in imidacloprid resistant *L. decemlineata* [130] and at
524 least one has been shown to have phenotypic effects on resistance traits following RNAi
525 knockdown [131]. A total of 163 putative cuticle protein genes were identified and assigned to
526 one of seven families (CPR, CPAP1, CPAP3, CPF, CPCFC, CPLCG, and TWDL)
527 (**Supplemental Figure 11S** and **Table 22S**). Similar to other insects, the CPR family, with the
528 RR-1 (soft cuticle), RR-2 (hard cuticle), and unclassifiable types, constituted the largest group of
529 cuticle protein genes (132) in the *L. decemlineata* genome. While the number of genes in *L.*
530 *decemlineata* is slightly higher than in *T. castaneum* (110), it is similar to *D. melanogaster* (137)
531 [132]. Numbers in the CPAP1, CPAP3, CPF, and TWDL families were similar to other insects,
532 and notably no genes with the conserved sequences for CPLCA were detected in *L.*
533 *decemlineata*, although they are found in other Coleoptera.

534 A total of 89 CYP (P450) genes were identified in the *L. decemlineata* genome, an
535 overall decrease relative to *T. castaneum* (143 genes). Due to their role in insecticide resistance
536 in *L. decemlineata* and other insects [38,48,133], we examined the CYP6 and CYP12 families in
537 particular. Relative to *T. castaneum*, we observed reductions in the CYP6BQ, CYP4BN, and
538 CYP4Q subfamilies. However, five new subfamilies (CYP6BJ, CYP6BU, CYP6E, CYP6F and
539 CYP6K) were identified in *L. decemlineata* that were absent in *T. castaneum*, and the CYP12
540 family contains three genes as opposed to one gene in *T. castaneum* (CYP12h1). We found
541 several additional CYP genes not present in *T. castaneum*, including CYP413A1, CYP421A1,
542 CYP4V2, CYP12J and CYP12J4. Genes in CYP4, CYP6, and CYP9 are known to be involved
543 in detoxification of plant allelochemicals as well as resistance to pesticides through their

544 constitutive overexpression and/or inducible expression in imidacloprid resistant *L. decemlineata*
545 [48,130].

546 GSTs have been implicated in resistance to organophosphate, organochlorine and
547 pyrethroid insecticides [134] and are responsive to insecticide treatments in *L. decemlineata*
548 [130,135]. A total of 27 GSTs were present in the *L. decemlineata* genome, and while they
549 represent an expansion relative to *A. glabripennis,* all have corresponding homologs in *T.*
550 *castaneum.* The cytosolic GSTs include the epsilon (11 genes), delta (5 genes), omega (4 genes),
551 theta (2 genes) and sigma (3 genes) families, while two GSTs are microsomal (**Supplementary**
552 **Figure 12S**). Several GST-like genes present in the *L. decemlineata* genome represent the Z
553 class previously identified using transcriptome data [135].

554

555 **Pest Control via the RNAi Pathway**

556 RNA interference (RNAi) is the process by which small non-coding RNAs trigger
557 sequence-specific gene silencing [136], and is important in protecting against viruses and mobile
558 genetic elements, as well as regulating gene expression during cellular development [137]. The
559 application of exogenous double stranded RNA (dsRNA) has been exploited as a tool to suppress
560 gene expression for functional genetic studies [138,139] and for pest control [41,140].

561 We annotated a total of 49 genes associated with RNA interference, most of them were
562 found on a single scaffold. All genes from the core RNAi machinery (from all three major RNAi
563 classes) were present in *L. decemlineata*, including fifteen genes encoding components of the
564 RNA Induced Silencing Complex (RISC) and genes known to be involved in double-stranded
565 RNA uptake, transport, and degradation **(Supplementary Table 22S).** A complete gene model
566 was annotated for *R2D2*, an essential component of the siRNA pathway that interacts with *dicer-*

2 to load siRNAs into the RISC, and not previously detected in the transcriptome of the *L. decemlineata* mid-gut [141]. The core components of the small interfering RNA (siRNA) pathway were duplicated, including *dicer-2*, an RNase III enzyme that cleaves dsRNAs and pre-miRNAs into siRNAs and miRNAs respectively [142,143]. The *dicer-2a* and *dicer-2b* CDS have 60% nucleotide identity to each other, and 56% and 54% identity to the *T. castaneum dicer-2* homolog, respectively. The *argonaute-2* gene, which plays a key role in RISC by binding small non-coding RNAs, was also duplicated. A detailed analysis of these genes will be necessary to determine if the duplications provide functional redundancy. The duplication of genes in the siRNA pathway may play a role in the high sensitivity of *L. decemlineata* to RNAi knockdown [144] and could benefit future efforts to develop RNAi as a pest management technology.

Conclusion

The whole-genome sequence of *L. decemlineata*, provides novel insights into one of the most diverse animal taxa, Chrysomelidae. It is amongst the largest beetle genomes sequenced to date, with a minimum assembly size of 640 Mb (ranging up to 1.17 Gb) and 24,740 genes. The genome size is driven in part by a large number of transposable element families, which comprise at least 17% of the genome and appear to be rapidly expanding relative to other beetles. Population genetic analyses suggest high levels of nucleotide diversity, local geographic structure, and evidence of recent population growth, which helps to explain how *L. decemlineata* rapidly evolves to exploit novel host plants, climate space, and overcome a range of pest management practices (including a large and diverse number of insecticides). Digestive enzymes, in particular the cysteine peptidases and carbohydrate-active enzymes, show evidence of gene expansion and elevated expression in gut tissues, suggesting the diversity of the genes is

a key trait in the beetle's phytophagous lifestyle. Additionally, expansions of the gustatory receptor subfamily for bitter tasting might be a key adaptation to exploiting hosts in the nightshade family, Solanaceae, while expansions of novel subfamilies of CYP and GST proteins are consistent with rapid, lineage-specific turnover of genes implicated in *L. decemlineata*'s capacity for insecticide resistance. Finally, *L. decemlineata* has interesting duplications in RNAi genes that might increase its sensitivity to RNAi and provide a promising new avenue for pesticide development. The *L. decemlineata* genome promises new opportunities to investigate the ecology, evolution, and management of this species, and to leverage genomic technologies in developing sustainable methods of pest control.

Materials and Methods

Genome Characteristics and Sampling of DNA and RNA

Previous cytological work determined that *L. decemlineata* is diploid and consists of 34 autosomes plus an XO system in males, or an XX system in females [145]. Twelve chromosomes are submetacentric, while three are acrocentric and two are metacentric, although one chromosome is heteromorphic (acrocentric and/or metacentric) in pest populations [146]. The genome size has been estimated with Feulgen densitometry at 0.46 pg, or approximately 460 Mb [47]. To generate a reference genome sequence, DNA was obtained from a single adult female, sampled from an imidacloprid resistant strain developed from insects collected from a potato field in Long Island, NY. Additionally, whole-body RNA was extracted for one male and one female from the same imidacloprid resistant strain. Raw RNAseq reads for 8 different populations were obtained from previous experiments: two Wisconsin populations (PRJNA297027) [130], a Michigan population (PRJNA400685), a lab strain originating from a

New Jersey field population (PRJNA275431), and three samples from European populations (PRJNA79581 and PRJNA236637) [43,121]. All RNAseq data came from pooled populations or were combined into a population sample from individual reads. In addition, RNA samples of an adult male and female from the same New Jersey population were sequenced separately using Illumina HiSeq 2000 as 100 bp paired end reads (deposited in the GenBank/EMBL/DDBJ database, PRJNA275662), and three samples from the mid-gut of 4th-instar larvae were sequenced using SOLiD 5500 Genetic Analyzer as 50 bp single end reads (PRJNA400633).

Genome Sequencing, Assembly, Annotation and Assessment

Four Illumina sequencing libraries were prepared, with insert sizes of 180 bp, 500 bp, 3 kb, and 8 kb, and sequenced with 100 bp paired-end reads on the Illumina HiSeq 2000 platform at estimated 40x coverage, except for the 8kb library, which was sequenced at estimated 20x coverage. ALLPATHS-LG v35218 [49] was used to assemble reads. Two approaches were used to scaffold contigs and close gaps in the genome assembly. The reference genome used in downstream analyses was generated with ATLAS-LINK v1.0 and ATLAS GAP-FILL v2.2 (https://www.hgsc.bcm.edu/software/). In the second approach, REDUNDANS was used [57], as it is optimized to deal with heterozygous samples. The raw sequence data and *L. decemlineata* genome have been deposited in the GenBank/EMBL/DDBJ database (Bioproject accession PRJNA171749, Genome assembly GCA_000500325.1, Sequence Read Archive accessions: SRX396450- SRX396453). This data can be visualized, along with gene models and supporting data, at the i5k Workspace@NAL: https://i5k.nal.usda.gov/Leptinotarsa_decemlineata and https://apollo.nal.usda.gov/lepdec/jbrowse/ [147]. We estimated the size of our genome using a

kmer distribution plot in JELLYFISH [148], where we mapped the 100 bp paired-end reads from the 180 bp insert library, used a 19 bp kmer distribution plot, and corrected for ploidy.

Automated gene prediction and annotation were performed using MAKER v2.0 [149], using RNAseq evidence and arthropod protein databases. The MAKER predictions formed the basis of the first official gene set (OGS v0.5.3). To improve the structural and functional annotation of genes, these gene predictions were manually and collaboratively edited using the interactive curation software Apollo [150]. For a given gene family, known insect genes were obtained from model species, especially *T. castaneum* [51] and *D. melanogaster* [151], and the nucleotide or amino acid sequences were used in *blastx* or *tblastn* [152,153] to search the *L. decemlineata* OGS v0.5.3 or genome assembly, respectively, on the i5k Workspace@NAL. All available evidence (AUGUSTUS, SNAP, RNA data, etc.), including additional RNAseq data not used in the MAKER predictions, were used to inspect and modify gene predictions. Changes were tracked to ensure quality control. Gene models were inspected for quality, incorrect splits and merges, internal stop codons, and gff3 formatting errors, and finally merged with the MAKER-predicted gene set to produce the official gene set (OGS v1.0; merging scripts are available upon request). For focal gene families (e.g. peptidase genes, odorant and gustatory receptors, RNAi genes, etc.), details on how genes were identified and assigned names based on functional predictions or evolutionary relationship to known reference genes are provided in the Supplementary Material (**Supplementary File**).

To assess the quality of our genome assemblies, we used BUSCO v2.0 [59] to determine the completeness of each genome assembly and the official gene set (OGS v1.0), separately. We benchmarked our data against 35 insect species in the Endopterygota obd9 database, which consists of 2,442 single-copy orthologs (BUSCOs). Secondly, we annotated and examined the

genomic architecture of the Hox and Iroquois Complex gene clusters. For this, tBLASTn searches were performed against the genome using orthologous Hox gene protein sequences from *T. castaneum* (Tcas3.0) and *A. glabripennis*. Provisional *L. decemlineata* models were refined, and potential gene duplications were identified, via iterative and reciprocal BLAST and by manual inspection and correction of protein alignments generated with ClustalW2 [154], using RNAseq expression evidence when available. Finally, we used BlobTools v1.0 [155] to assess the assembled genome for possible contamination by generating a Blobplot. This plot integrates guanine and cytosine (GC) content of sequences, read coverage and sequence similarity via blast searches to assess genome contamination. Putative contaminants were identified using DIAMOND BLAST [156] to query the genome scaffolds against the UniProt reference database [157], and read coverage was assessed by mapping the 100 bp paired-end reads from the 180 bp insert library to the ALLPATHS genome with the Burrows-Wheeler Aligner (bwa-mem) version 0.7.5a [158].

Gene Family Evolution

In order to identify rapidly evolving gene families along the *L. decemlineata* lineage, we obtained ~38,000 ortho-groups from 72 Arthropod species as part of the i5k pilot project (Thomas et al. *in prep*) from OrthoDB version 8 [159]. For each ortho-group, we took only those genes present in the order Coleoptera, which was represented by the following six species: *A. glabripennis*, *A. planipennis*, *D. ponderosae*, *L. decemlineata*, *O. taurus*, and *T. castaneum* (http://i5k.github.io/) [51,52,54]. Finally, in order to make accurate inferences of ancestral states, families that were present in only one of the six species were removed. This resulted in a final count of 11,598 gene families that, among these six species, form the comparative

framework that allowed us to examine rapidly evolving gene families in the *L. decemlineata* lineage.

Aside from the gene family count data, an ultrametric phylogeny is also required to estimate gene gain and loss rates. To make the tree, we considered only gene families that were single copy in all six species and that had another arthropod species also represented with a single copy as an outgroup. Outgroup species were ranked based on the number of families in which they were also single copy along with the coleopteran species, and the highest ranking outgroup available was chosen for each family. For instance, *Pediculus humanus* was the most common outgroup species. For any gene family, we chose *P. humanus* as the outgroup if it was also single copy. If it was not, we chose the next highest ranking species as the outgroup for that family. This process resulted in 3,932 single copy orthologs that we subsequently aligned with PASTA [160]. We used RAxML [161] with the PROTGAMMAJTTF model to make gene trees from the alignments and ASTRAL [162] to make the species tree. ASTRAL does not give branch lengths on its trees, a necessity for gene family analysis, so the species tree was again given to RAxML along with a concatenated alignment of all one-to-one orthologs for branch length estimation. Finally, to generate an ultrametric species tree with branch lengths in millions of years (my) we used the software r8s [163], with a calibration range based on age estimates of a crown Coleopteran fossil at 208.5-411 my [164]. This calibration point itself was estimated in a similar fashion in a larger phylogenetic analysis of all 72 Arthropod species (Thomas et al. *in prep*).

With the gene family data and ultrametric phylogeny as the input data, gene gain and loss rates (λ) were estimated with CAFE v3.0 [165]. CAFE is able to estimate the amount of assembly and annotation error (ε) present in the input data using a distribution across the

704 observed gene family counts and a pseudo-likelihood search, and then is able to correct for this
705 error and obtain a more accurate estimate of λ. Our analysis had an ε value of about 0.02, which
706 implies that 3% of gene families have observed counts that are not equal to their true counts.
707 After correcting for this error rate, $\lambda = 0.0010$ is on par with those previously those found for
708 other Arthropod orders (Thomas et al. *in prep*). Using the estimated λ value, CAFE infers
709 ancestral gene counts and calculates p-values across the tree for each family to assess the
710 significance of any gene family changes along a given branch. Those branches with low p-values
711 are considered rapidly evolving.
712
713 ***Gene Expression Analysis***
714 RNAseq analyses were conducted to 1) establish male, female, and larva-enriched gene
715 sets and 2) identify specific genes that are enriched within the digestive tract (mid-gut) compared
716 to entire larva. RNAseq datasets were trimmed with CLC Genomics v.9 (Qiagen) and quality
717 was assessed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Each
718 dataset was mapped to the predicted gene set (OGS v1.0) using CLC Genomics. Reads were
719 mapped with >90% similarity over 60% of length, with two mismatches allowed. The number of
720 reads were corrected to reads per million mapped to allow comparison between RNAseq datasets
721 that having varying coverage. Transcripts per million (TPM) was used as a proxy for gene
722 expression and fold changes were determined as the TPM in one sample relative to the TPM of
723 another dataset [166]. The Baggerly's test (t-type test statistic) followed by Bonferroni correction
724 was used to identify genes with significant enrichment in a specific sample [167]. Statistical
725 values for Bonferroni correction were reported as the number of genes x α value. This stringent
726 statistical analysis was used as only a single replicate was available for each treatment. Enriched

727 genes were removed, and mapping and expression analyses were repeated to ensure low
728 expressed genes were not missed. Genes were identified by BLASTx searching against the NCBI
729 non-redundant protein databases for arthropods with an expectation value (E-value) < 0.001.
730
731 ***Transposable Elements***
732 We investigated the identities (family membership), diversity, and genomic distribution
733 of active transposable elements within *L. decemlineata* in order to understand their contribution
734 to genome structure and to determine their potential positional effect on genes of interest
735 (focusing on a TE neighborhood size of 1 kb). To identify TEs and analyze their distribution
736 within the genome, we developed three repeat databases using: 1) RepeatMasker [168], which
737 uses the library repeats within Repbase (http://www.girinst.org/repbase/), 2) the program
738 RepeatModeler [169], which identifies de-novo repeat elements, and 3) literature searches to
739 identify beetle transposons that were not found within Repbase. The three databases were used
740 within RepeatMasker to determine the overall TE content in the genome.
741 To eliminate false positives and examine the genome neighborhood surrounding active
742 TEs, all TE candidate models were translated in 6 frames and scanned for protein domains from
743 the Pfam and CDD database (using the software transeq from Emboss, hmmer3 and rps-blast).
744 The protein domain annotations were manually curated in order to remove: a) clear false
745 positives, b) old highly degraded copies of TEs without identifiable coding potential, and c) the
746 correct annotation when improper labels were given. The TE models that contained protein
747 domains were mapped onto the genome and used for our neighborhood analysis: we extracted
748 the 1 kb flanking regions for each gene and scanned these regions for TEs with intact protein
749 coding domains.

Population Genetic Variation and Demographic Analysis

Population genetic diversity of pooled RNAseq samples was used to examine genetic structure of pest populations and past population demography. For Wisconsin, Michigan and the lab strains from New Jersey, we aligned the RNAseq data to the genomic scaffolds, using Bowtie2 version 2.1.0 [170] to index the genome and generate aligned SAM files. We used bwa [150] to align the RNAseq from the three populations from Europe. SAMtools/BCFtools version 0.1.19 [171] was used to produce BAM and VCF files. All calls were filtered with VCFtools version 0.1.11 [172] using a minimum quality score of 30 and minimum depth of 10. All indels were removed from this study. Population specific VCF files were sorted and merged using VCFtools, and the allele counts were extracted for each SNP. These allele frequency data were then used to infer population splits and relative rates of genetic drift using Treemix version 1.12 [173]. We ran Treemix with SNPs in groups of 1000, choosing to root the tree with the Wisconsin population. In addition, for each pair of populations, we estimated the average genetic divergence by using F-statistics (F_{ST}) in VCFtools to calculate the ratio of among- to within-population genetic divergence across SNP loci.

To infer patterns of demographic change in the Midwestern USA (Wisconsin and Michigan) and European populations, the genome-wide allele frequency spectrum was used in *dadi* version 1.6.3 [174] to infer demographic parameters under several alternative models of population history. The history of *L. decemlineata* as a pest is relatively well-documented. The introduction of *L. decemlineata* into Europe in 1914 [175] is thought to have involved a strong bottleneck [15] followed by rapid expansion. Similarly, an outbreak of *L. decemlineata* in Nebraska in 1859 is thought to have preceded population expansion into the Midwest reaching

773 Wisconsin in 1865 [3]. For each population, a constant-size model, a two-epoch model of
774 instantaneous population size change at a time point τ, a bottle-growth model of instantaneous
775 size change followed by exponential growth, and a three-epoch model with a population size
776 change of fixed duration followed by exponential growth, was fit to infer θ, the product of the
777 ancestral effective population size and mutation rate, and relative population size changes.
778

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SZ contributed additional RNAseq data. JBB coordinated the RNAseq data analysis. KB, AM, and YHC conducted the transposable element analysis. SDS and JC conducted the population genetics analysis.

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Figure Legend

Figure 1. Ultrametric tree with branch lengths in millions of years for *Leptinotarsa decemlineata* relative to five other Coleoptera genomes. The *L. decemlineata* lineage is shown in orange. Branches are labeled with their length in years (top) and with the number of gene family expansions (blue) and contractions (purple) that occurred on that lineage. Rapid changes for both types are in parentheses.

Figure 2. Heatmap distribution of the abundance of transcription factor families in *Leptinotarsa decemlineata* compared to other insects. Each entry indicates the number of TF genes for the given family per genome, based on presence of predicted DNA binding domains. Color scale is

log (base 2) and the key is depicted at the top (light blue means the TF family is completely absent). Families discussed in the main text are indicated by arrows.

Figure 3. Volcano plots showing statistically significant gene expression differences after Bonferroni correction in *Leptinotarsa decemlineata*. A) Mid -gut tissue versus whole larvae, B) an adult male versus an adult female, C) an adult male versus whole larvae, and D) an adult female versus whole larvae. Points outside the gray area indicate >100-fold-differences in expression. Blue points indicate down-regulated genes and red points indicate up-regulated genes in each contrast.

Figure 4. Population genetic relationships and relative rates of genetic drift among *Leptinotarsa decemlineata* pest populations based on single nucleotide polymorphism data. Population codes: NJ- New Jersey lab strain, WIs- imidacloprid susceptible population from Arlington, Wisconsin, WIr- imidacloprid resistant population from Hancock, Wisconsin, MI- imidacloprid resistant population from Michigan, and EU- European samples combined from Italy and Russia.

Figure 5. Phylogenetic relationships of the cysteine peptidase gene family in *Leptinotarsa decemlineata* compared to model insects. Species abbreviations are: *L. decemlineata* (Ld, green color), *Drosophila melanogaster* (Dm, blue color), *Apis mellifera* (Am, purple color), and *Tribolium castaneum* (Tc, red color). Mid-gut gene expression (TPM) of highly expressed *L. decemlineata* cysteine peptidases is shown as bar graphs across three replicate treatments.

1272 **Table 1:** Summary of gene gain and loss events inferred after correcting for annotation and
1273 assembly error across six Coleoptera species. The number of rapidly evolving families is shown
1274 in parentheses for each type of change and the rate is genes per million years.

	Expansions			Contractions			No Change	Average Expansion
	Families	Genes gained	Rate	Families	Genes lost	Rate		
Anoplophora glabripennis	865 (13)	1231	1.42	1988 (107)	3125	1.57	5850	-0.182341
Agrilus plannipennis	739 (100)	1991	2.69	707 (8)	769	1.09	7257	0.119108
Dendroctonus ponderosae	933 (72)	1982	2.12	1606 (21)	1887	1.17	6164	0.006438
Leptinotarsa decemlineata	426 (40)	855	2.01	1556 (48)	2116	1.36	6721	-0.127501
Onthophagus taurus	1299 (142)	2952	2.27	767 (24)	895	1.17	6637	0.203380
Tribolium castaneum	786 (51)	1428	1.82	516 (27)	909	1.76	7401	0.055645

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1276 **Table 2.** Numbers of putatively functional proteins in four chemosensory families in
1277 *Leptinotarsa decemlineata* and other beetle species.

Species	Odorant Binding Proteins (OBPs)	Olfactory Receptors (ORs)	Gustatory Receptors (GRs)	Ionotropic Receptors (IRs)
Leptinotarsa decemlineata	58	75	144	27
Anoplophora glabripennis	52	120	234	72
Tribolium castaneum	49	264	219	72

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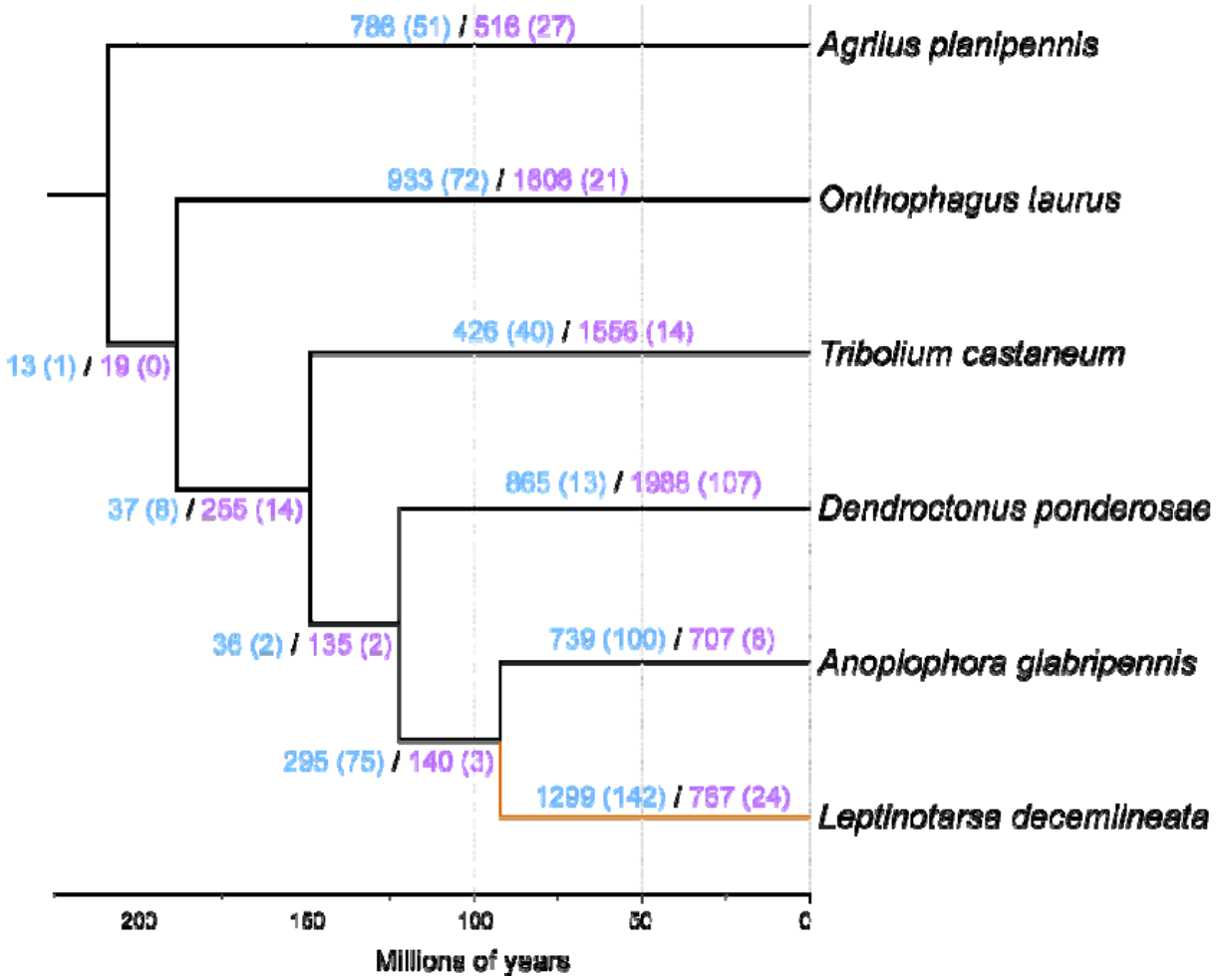

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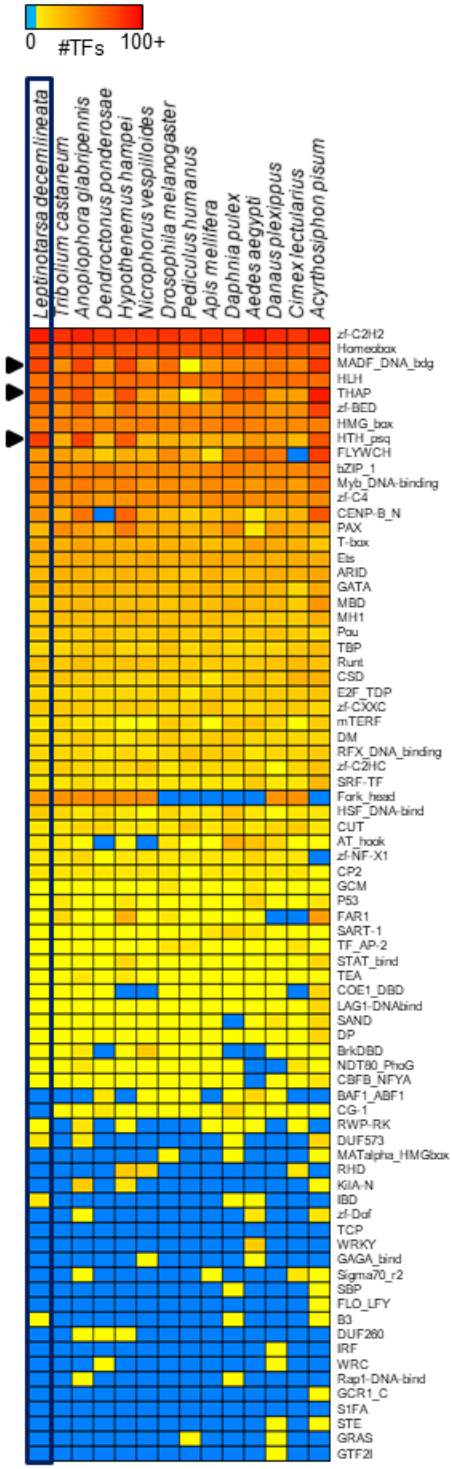

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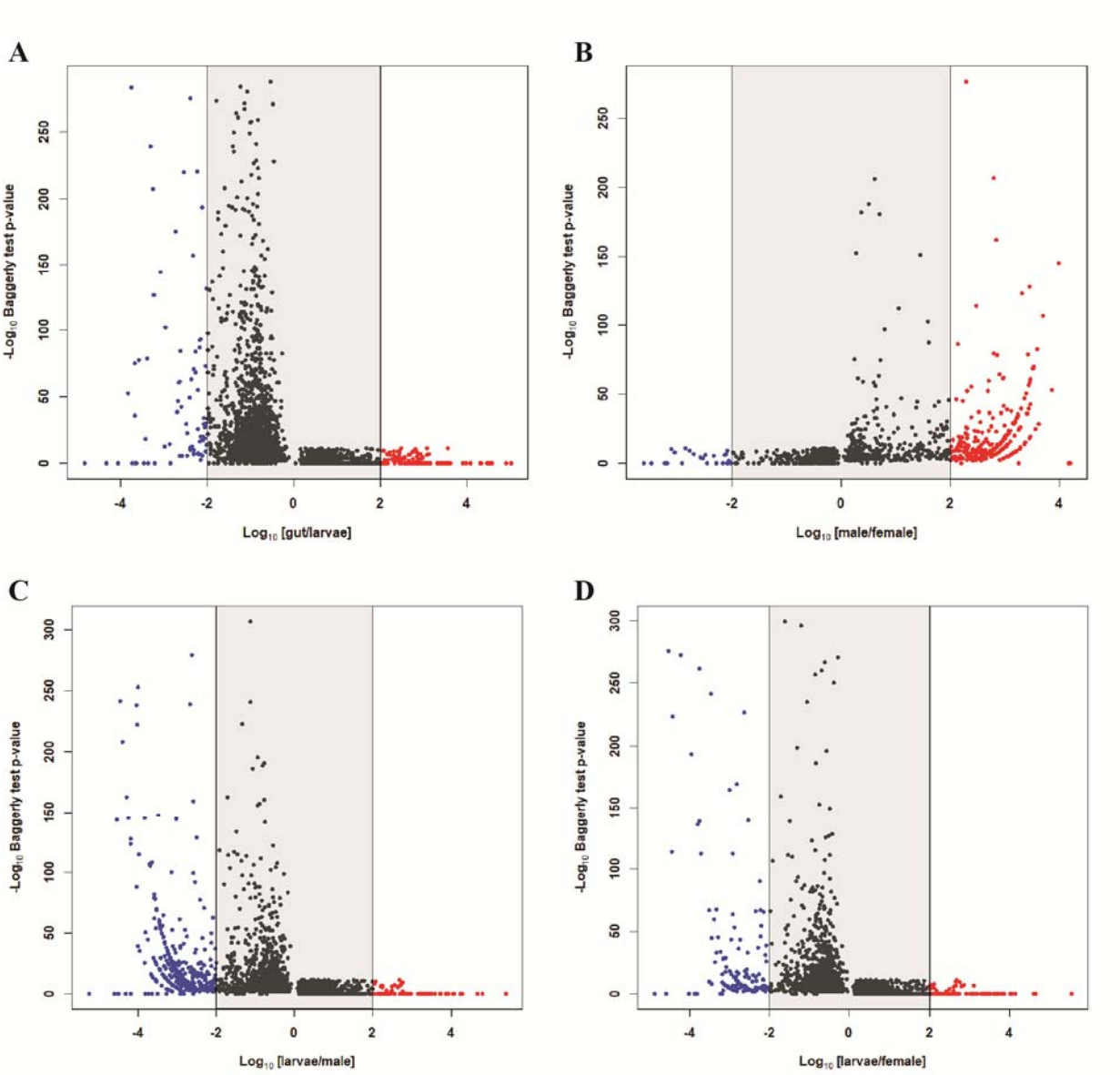

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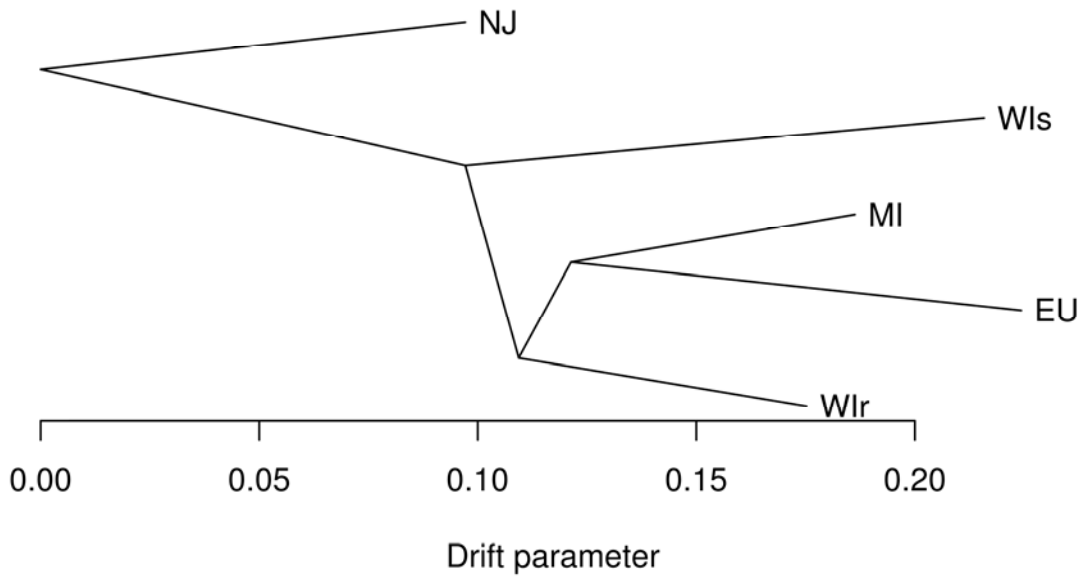

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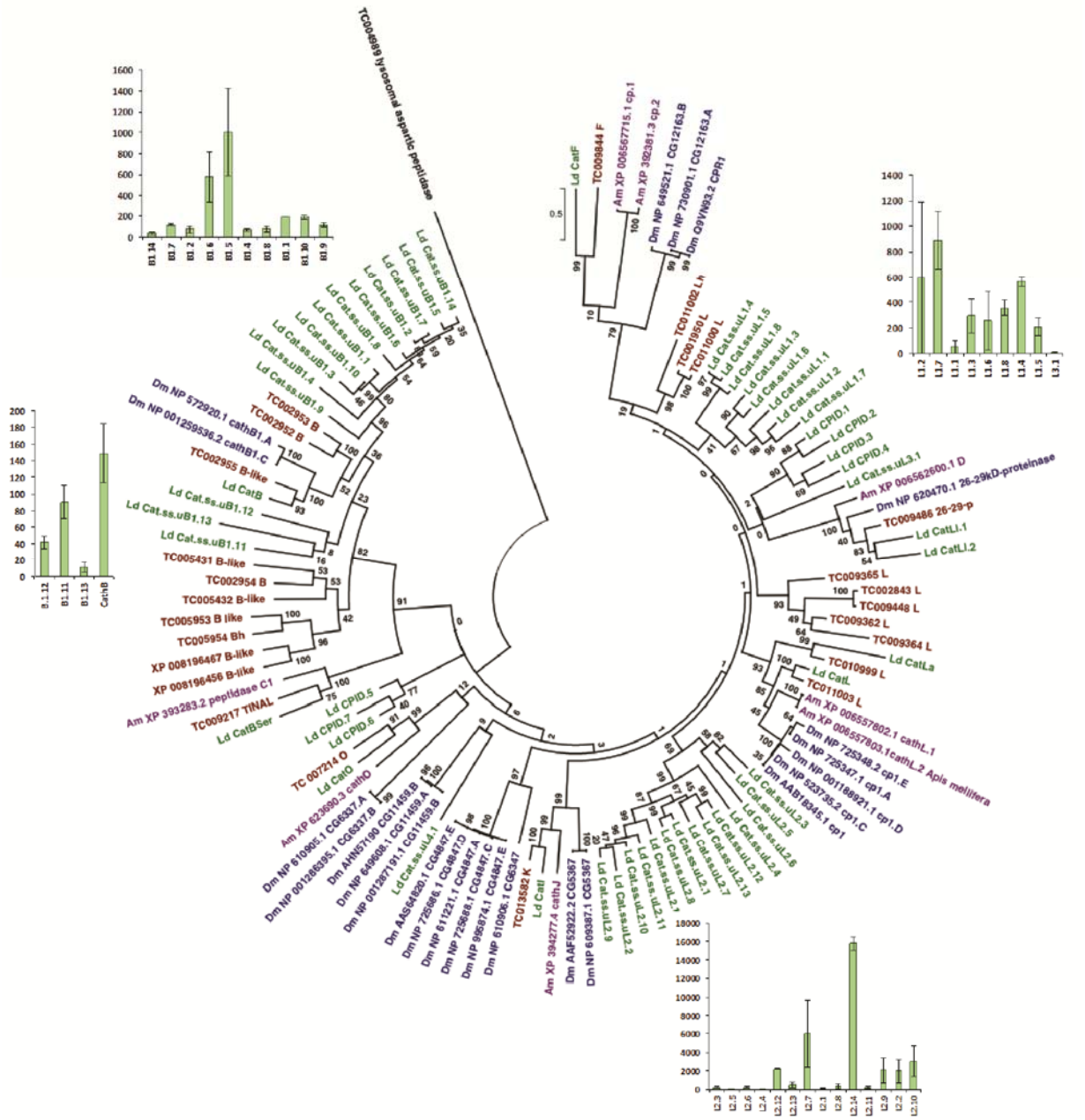

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Supplementary Files

Supplementary File.pdf Supplementary Materials. Contains additional methods, results, figures and tables.

Supplementary Dataset 1. Precursor miRNA nucleotide sequences.

Supplementary Dataset 2. miRNA predictions (gff format).

Supplementary Dataset 3. Peptide sequences of annotated olfactory genes. Includes the Odorant Binding Proteins (OBPs), Odorant Receptors (ORs), Gustatory Receptors (GRs), and Ionotropic Receptors (IRs). The IRs from *Tribolium castaneum* are also included.