Review

Arthropod genomics research in the United States Department of Agriculture-Agricultural Research Service: Current impacts and future prospects

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ABSTRACT

The Agricultural Research Service (ARS) is the intramural research agency of the United States Department of Agriculture (USDA) which employs scientists to conduct basic and applied research for the development and transfer of solutions to agricultural problems of high national priority and to ensure food security for domestic and export needs. Economically important and invasive arthropods, especially insect, tick, and mite species, negatively impact the health of every plant and animal, resulting in significant production losses among crop, livestock and forestry commodities amounting to billions of US dollars annually. The economic impact increases further when the insect damage to home and urban landscapes is taken into account. Ensuring continued viability of beneficial insects in the environment is crucial for the pollination of all horticultural and orchard crops, as well as for enhancing natural biological control of pest arthropods and overall ecosystem sustainability. Mitigating damage by arthropod pest species and safeguarding the health of beneficial arthropods are key components for ensuring the stability of agricultural production in the United States and abroad. Developments in genomics, including next generation sequencing (NGS), provide a suite of new research tools within applied agricultural arthropod research. Contributions by the ARS to arthropod genomics research are reviewed herein. These research approaches are discussed briefly in the context of improving agricultural production and environmental stewardship. Arthropod genomics research within ARS is contributing significantly to the improvement of agricultural production and environmental stewardship for farmers, the agricultural industry, and consumers alike.

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1. Introduction

Arthropods are the largest animal group in the world, representing greater than 75% of all described species, and occupying almost every conceivable ecological niche. Arthropods, especially insects, attack every single plant and animal of agricultural importance, and damage commodities ranging from grains, fibers, biofuels, fruits, vegetables, ornamentals and forestry, to livestock. The importance of arthropod pest control is highlighted in the setting of 'managing new pests, pathogens, and invasive plants' as a top agricultural challenge for the 21st century by the President's Council of Advisors on Science and Technology [1]. Potential yield losses in lieu of crop protection is estimated to be greater than 50%, and is a major contributor to human malnourishment and starvation [2]; realized losses even in developed countries approach nearly 18% [3]. The damage that arthropods inflict on cultivated plants is highly varied, and includes direct feeding on leaves, fiber, grain and fruits, facilitating the entry of pathogens or vectoring pathogens that cause plant diseases. Impacts on plant health effectively reduce plant nutrient uptake and energy production, tolerance to other biotic factors such as drought, and structural support by causing root, stalk, stem and trunk damage. Arthropods also affect animal health by acting as parasites, causing allergenic response and blood loss, or by vectoring disease agents [4], which can dramatically impact the efficiency of livestock production by reducing reproductive, growth and survival rates [5-7]. The economic impact of arthropod pests can be measured by the loss of market value of the products they destroy, the costs to repair the damage they inflict, the infectious agents some species transmit to plants or animals, and the resources expended on prevention and control (Tables 1 and 2). When one considers the hundreds of major arthropod pests, the annual economic losses to agriculture exceed US$100 billion. Moreover, human health and other non-target arthropods may be negatively impacted by the practices used to control agricultural pest species [8, 9]. Many non-target beneficial arthropods are important to agricultural and natural systems by providing pollination, controlling weeds, and regulating the growth of pest species populations.

The molecular biology revolution of the 1980s and 1990s allowed the study of genes and genome regions one-by-one by applying advances in polymerase chain reaction (PCR), cloning, and Sanger DNA sequencing. While the throughput of these initial methods was low and costs were high, the rewards were great following the dramatically scaled up Sanger sequencing efforts that resulted in the assembly of the human genome at a cost of approximately US$2.7 billion. Several arthropod genomes of significantly smaller size were also assembled from Sanger sequence data during this era, including the fruit fly, *Drosophila melanogaster* [10], honey bee, *Apis mellifera* [11], and mosquito, *Anopheles gambiae* [12]. The subsequent development of next generation sequencing (NGS) technologies in the early 2000s represented a huge breakthrough that initiated a paradigm shift in the biological sciences [13]. Specifically, NGS technologies now allow the generation of many-fold greater amounts of nucleotide sequence data at lower expense and reduced time compared with traditional Sanger sequencing approaches [14]. Due to these NGS technologies, the generation of nucleotide sequence data has become less inhibitory and the application of genomic-scale studies is now possible on virtually all organisms. These NGS applications have resulted in both genome assemblies, and the identification and quantification of expressed gene transcripts in various species [15]. NGS methods for genetic marker discovery within short read data from reduced representation libraries of barcoded individuals also were developed through Restriction site Associated DNA sequencing (RAD-seq) [16] and genotyping-by-sequencing (GBS) approaches [17]. These new methods also allow researchers to more reliably estimate genetic variation in populations and genome-wide associations between traits and causal genes [18]. While genomics is a discipline in and of itself, with a defined set of concepts, procedures and analytical pipelines, the true power of genomics lies in the applications that address basic fundamental and translational research. This not only entails laboratory expertise and appropriate experimental designs, but also the development of computational resources required to analyze the ever growing amounts of NGS data.

Table 1. Economic impact of selected arthropod pest species to crop production.

Cotton pest insects and mites cause estimated economic damage of more than $1.3 billion US dollars per year when considering changes in cotton production, prices, processing and use of other commodities [222].
Vegetable crop damage by the major pest, the diamondback moth, conservatively inflict US$4 billion in management costs to producers annually [223].
Potato, eggplant and tomato crops are extensively defoliated by the Colorado potato beetle, which causes annual crop losses and the need for pest control measures that cost nearly US$150 million – even though this beetle has thwarted control efforts by evolving resistance to almost every contemporary class of pesticide chemistries [224].
Corn is the most widely cultivated crop in the US, to which the Western corn rootworm inflicts feeding damage on corn roots as larvae and reproductive tissue as adults. This plant damage causes reduced plant nutrient uptake and instability, and can significantly decrease grain production during heavy infestations. Adaptation to multiple control practices including crop rotations, chemical insecticides and transgenic corn has led to several major corn growing regions, wherein the total losses are estimated in excess of US$1.17 billion in the United States alone [42].
Citrus crops in the states of Florida and California respectively incur US$4.5 billion and US$2.7 billion annually due to citrus greening disease (or Huanglongbing) largely transmitted by the Asian citrus psyllid [225].
The annual economic impact of fire ants is more than US$6.5 billion dollars annually in the United States. Additional economic losses associated with the potential spread of fire ants throughout California and Hawaii are US$900 million and US$211 million, respectively [226-228].
Wine grapes infected by Pierce's disease, which is caused by an insect-transmitted bacteria, costs growers and consumers US$61 million annually. If this non-native vector were to spread throughout California, the annual cost to the wine grape industry would increase by US$261 million [229].

Table 2. Economic impact of selected arthropod pest species on livestock production.

The stable fly is a pest of cattle on pasture and range that causes estimated economic damage of $2.2 billion US dollars per year [234].
Horn flies are a serious pest of cattle in the Americas that affects the productivity of livestock operations by interfering with normal feeding activity, causing loss of blood, reduced weight gains, and decreased milk production in infested cows, which in the U.S. translates into economic loss to producers estimated to exceed $800 million US dollars annually [235].
Around 80 tick species are present in the U.S. and several of them are of economic relevance in animal agriculture because of their obligate blood feeding habit and in some cases the ability to transmit disease-causing agents some of which can affect humans too like the Lyme disease spirochete [83]; previous estimates converted to today's currency rate indicate that losses attributable to ticks affecting livestock total approximately $1 billion US dollars annually considering the cost of acaricide treatment [236].

The fields of bioinformatics and computational biology grew from the development of methods to analyze NGS data, as well as the requirement of biological research to store, organize and process 'big data' [19].

United States Department of Agriculture, Agricultural Research Service (USDA-ARS) scientists conduct applied translational research to control arthropod pests to meet the needs of commodity producers, processors and consumers. Delivery of solutions to stakeholders remains the goal of ARS research, and the rising field of genomics has already contributed findings to help control pest arthropods in crop, livestock and forestry industries, as well as to enhance the well-being of beneficial insect pollinators and natural enemies. Collaborative research by the ARS also has helped solve problems in public health and to protect U.S. troops from

arthropod vectors [20, 21]. Herein, we provide a brief overview of ARS impacts from selected arthropod genomic studies. More specifically, research is briefly highlighted within four main areas, including: 1) management and control of arthropod pests affecting cultivated crops, 2) management and control of arthropod pests affecting livestock, 3) management and control of invasive arthropods, and 4) management and improvement of beneficial arthropod health. One or more ARS case studies are presented for each of these topics. We note that this is not comprehensive for all of the above topics, but rather is intended to showcase research trends in arthropod genomics at the ARS and to provide perspectives on potential future role of genomics in enhancing agricultural production.

2. Management and control of arthropod pests affecting cultivated crops

Large-scale intensive agriculture is a human achievement that includes technical advances that have increased productivity on a relatively fixed amount of arable land and improvements in mechanization, genetics, and nutrition and health. The "Green Revolution" of the 1960s ushered in an era wherein broadcast applications of chemical pesticides became the cornerstone of insect management practices, arguably, with the concomitant abandonment of traditional cultural control practices. Despite initial successes, this ubiquitous application of chemical pesticides across agroecosytems is implicated as a major factor that led to the evolution of functional resistance in insects. Evidence of this response to selection within multiple insect populations came from repeated instances of field-evolved resistance to organochlorine-, carbamate-, and organophosphate-based pesticide formulations. Classical models describing the molecular basis of chemical pesticide resistance include mutations in target proteins, mechanisms that increase the capacity for metabolic detoxification, and decreased cuticular absorption [22]. Early discoveries of receptor site mutations identified a single amino acid substitution in the gamma-aminobutyric acid (GABA) receptor that confers resistance to organochlorine insecticides [23]. Interestingly, this mutation has occurred multiple times in the same species such that

different populations of a single insect species can be assumed to have the potential to independently evolve resistance. Furthermore, it has been shown that this same GABA receptor amino acid change led to organochlorine insecticide resistance in several different insect species. Thus, similar or identical mutations that confer resistance have evolved independently within a species as well as among different insect species [24]. Evidence also exists demonstrating that different mutations in a single receptor molecule can give rise to resistance [25, 26]. And there are clear examples of insects developing resistance to multiple pesticides. For instance, the green peach-potato aphid, *Myzus persicae*, codling moth, *Cydia pomonella*, and corn rootworm, *Diabrotica virgifera virgifera*, can show resistance to multiple insecticide chemistries in the same individual [27-29].

More recently, transgenic crops were developed that express *Bacillus thuringiensis* (Bt) toxins by genetic engineering to express insecticidal toxins within growing plant tissues, but have experienced a similar fate as their chemical predecessors in the occurrence of field-evolved resistance within some insect populations [30]. One main difference between the implementation of Bt transgenic plants versus chemical pesticides was the *a priori* acknowledgement that resistance was likely to evolve in the former, and insect resistance management (IRM) plans were developed in attempts to delay this onset of resistance. These IRM plans used a "high-dose" refuge (HDR) strategy designed to maintain the overall susceptibility of insect populations in an effort to prolong or preserve the utility of Bt technologies [31-33]. Despite these efforts, IRM strategies for Bt crops have failed in multiple instances due to the influence of unanticipated genetic, biological and ecological complexities [34]. The potential for application of RNA interference (RNAi) technologies for insect pest control in maize has now been demonstrated [35], and shown to induce high levels of mortality in larval western corn rootworm, *D. v. virgifera* [36] following knockdown of transcripts of the Snf7 vacuolar sorting protein [37]. Interestingly, susceptibility of *D. v. virgifera* to RNAi suppression was subsequently shown to be variable across populations [38]. Although the efficacy of some current insect control strategies has been compromised, pesticides remain a viable

and effective option for farmers in many cropping systems. The reoccurrence of resistance to chemical and transgenic pesticides might indicate that there is a lack of sufficient biological, ecological or genetic data to formulate effective long-term IRM plans, or arguably, to even protect the durability of certain technologies in the short-term.

The propensity of pest arthropods to develop resistance to control tactics with a single mode of action applied across the agricultural landscape has led to the development of alternative approaches using Integrated Pest Management (IPM) strategies. A centerpiece of IPM is to combine the prudent use of chemical pesticides and biological toxins with biological control agents, host plant resistance, and cultural control tools to suppress pest populations. The elimination of producer reliance on a single arthropod pest control tactic and a shift towards a multifaceted approach is a proposed method that may circumvent the early onset of pesticide resistance [39]. Indeed, empirical evidence shows that predators and parasitoids of target insects can effectively delay the increase of Bt resistance allele frequencies in multi-generational greenhouse studies [40], and also reduce the overall fitness of homozygous resistant individuals [41].

Cultivated crops are major domestic and export commodities, and they provide raw materials for industrial production, and feed for livestock and direct human consumption. The impacts of arthropods on this agricultural sector are substantial (see Introduction), and applied genomic studies at ARS to advance scientific knowledge and provide innovations within grain, orchard and horticultural crop production systems are highlighted below.

2.1. Pesticide resistance in major pest insects of corn

The USDA-ARS Corn Insects & Crop Genetics Research Unit (CICGRU), located in Ames, IA, along with university collaborators, has identified the genomic basis of pesticide resistance for *D. v. virgifera* and the European corn borer, *Ostrinia nubilalis*. *Diabrotica v. virgifera* is a native species that is arguably the most important pest arthropod of corn in the United States in terms of economic impact due to yield loss and costs of producer control measures [42], and difficulty of control due to evolution of resistance to chemical pesticides [43-46], transgenic Bt plants [47, 48], as well as crop rotations [49, 50]. One contribution of CICGRU scientists to *D. v. virgifera* control included development of a single nucleotide polymorphism (SNP) marker that detects a GABA receptor mutation conferring cyclodiene organochlorine pesticide resistance, for direct monitoring of resistance in the field [51]. These ARS researchers also used cDNA microarrays to show a correlation between the up-regulation of a carboxyesterase and inheritance of organophosphate resistance (Coates *et al.*, 2015, in press). A draft *D. v. virgifera* genome assembly has been completed (H. Robertson, personal communication, which was supported by genome size and repetitive DNA composition estimates obtained at the CICGRU [52, 53]. This genome assembly will be a valuable resource for identifying genes involved in Bt resistance already identified in the field [47, 48].

Ostrinia nubilalis was accidentally introduced into the eastern United States in the early 1900s from central Europe, and it subsequently spread to major corn growing regions in the Midwest by the 1950s, and henceforth had caused major economic losses to producers until the advent of Bt transgenic corn hybrids [54]. HDR strategy-based IRM plans mandated by the United States Environmental Protection Agency (US-EPA) were largely based on the biology and population genetics data of *O. nubilalis* underpinned by CICGRU-led microsatellite and SNP marker based estimates of gene flow [55-57]. Some of these data indicated that gene flow and moth dispersal may be more limited in the Northeastern United States because of the regional topography and smaller, more geographically-dispersed cornfields. This scenario contrasts with the assumptions of the HDR strategy, where localized increases in resistance allele frequencies may not be effectively counteracted by an influx of susceptible alleles. Furthermore, populations in the Northeastern United States are composed of two biotypes that differ in the compositions of sex pheromones females emit to attract males (i.e., *E*- and *Z*-pheromone races). CICGRU scientists developed SNP markers in the pheromone gland fatty acyl-reductase genes capable of differentiating *E*- and *Z*-pheromone strains [58], greatly facilitating efforts

to estimate gene flow between strains and evaluate impacts on durability of IRM strategies. Despite the lack of field-evolved Bt resistance, *O. nubilalis* has become a laboratory model for uncovering the mechanisms by which Bt resistance can occur. For example, increased Cry1Ab tolerance in a CICGRU laboratory colony was shown to be inherited independent of known Bt binding receptors [59, 60], and shown to result from mutations in gene regulatory factors acting in *trans-* (i.e., as transcription factors) to modify the level of Bt toxin receptors in the midgut, and thus mediating Cry1Ab resistance [61]. Specifically, high throughput SNP genotyping identified genome regions on linkage group 23 (LG23) and LG27 that influenced the inheritance of Cry1Ab toxin resistance and also caused the reduced transcription of a Bt toxin binding receptor, aminopeptidase N1 (*apn*1), in the midgut. This study bolstered the premise that mutations in gene regulation can give rise to Bt toxin resistance, which was demonstrated previously with respect to Cry1Ac resistance in the cabbage looper [62]. Analogously, the CICGRU led research efforts that mapped a QTL for *O. nubilalis* Cry1Fa resistance using high density genotyping-by-sequencing (GBS) markers [63], and determined that an ABCC2 transporter may not be the causal locus in Lepidoptera as previously thought [64]. These results demonstrated the ability of genomic applications to produce a high density of genetic markers sufficient to resolve differences necessary for fine mapping of traits.

2.2. Genomics and transcriptomics of the Mediterranean fruit fly

The USDA-ARS, Insect Behavior and Biocontrol Research Unit (IBBRU), Gainesville, FL, studies Tephritid fruit fly pest species which are among the most devastating pests to agriculture globally. Control of these pests in the United States uses intensive and costly biological control programs; primary among these is the sterile insect technique (SIT). The SIT involves the release of overwhelming large numbers of sterile (usually male) insects, which can be augmented by predatory parasitoid releases [65]. The SIT generally uses irradiation for sterilization of males, which has several drawbacks including a reduction in male fitness compared with wild type males in the field. In the absence of separating or eliminating females early

in development, they must be reared, sterilized and released with the males increasing the costs and reducing efficiency of the SIT. It is also necessary to mark released males to assess program effectiveness, which is achieved by dusting pupae with fluorescent marker dyes, but this is unreliable and presents health hazards to workers in the rearing facilities. Thus, desired improvements in SIT include the development and use of germ-line transformation to introduce transgene cassettes for whole body and sperm-specific marking, and conditional lethal genes that are female- and non-sex-specifically regulated to facilitate genetic sexing (i.e., male-only strains) and male genetic sterility, respectively. Advancements have been made in these approaches for *Anastrepha* species at IBBRU [66, 67]; however, the widespread and long-term application of these molecular-based systems will depend on the discovery of analogous genetic elements in other species, especially the identification and characterization of lethal effecter gene products [68, 69]. To facilitate these needs, an international group of researchers led by scientists at ARS and the Baylor College of Medicine are engaged in a Medfly Whole Genome Sequencing Project, including RNA-seq-based transcriptomics. One objective of this project is to identify gene and regulatory sequences that control embryonic and general development, female and male reproduction, sex-determination, programmed cell death, chemoreception, insecticide resistance and detoxification, and general metabolism among other critical processes related to invasiveness and population control. For control programs, identified genetic elements are expected to be developed into transgenic temperature- and tetracycline-dependent conditional lethality strains for male-only sexing strains (by female-specific-lethality) and male sterility strains by spermatocyte-specific and embryonic lethality [67, 70, 71]. Insertion of these genes into transgenic lines may be facilitated by targeted transformations at defined genomic loci, such that random insertional mutations with detrimental phenotypes and suppressive genomic position effects may be avoided [72, 73]. More generally, increased fundamental knowledge of medfly genetics and biology through structural and functional genomics studies will facilitate improvements and expansion of integrated pest management strategies for this and related tephritid pest species.

2.3. Control of whitefly-transmitted plant viruses

ARS scientists from the United States Vegetable Laboratory (USVL) in Charleston, SC and the Agricultural Research Station in Salinas, CA, in cooperation with a scientist at the Boyce Thompson Institute for Plant Research (BTI) at Cornell University, Ithaca, NY, have developed an area-wide project to control whitefly (*Bemisia tabaci*) and its transmission of plant viruses. Whiteflies are efficient vectors in transmitting a number of economically important plant viruses in the genera of *Begomovirus*, *Carlavirus*, *Crinivirus*, *Torradovirus*, and *Ipomovirus*. Whitefly transmitted viruses have become a major threat to global crop production, such as *Tomato yellow leaf curl virus* (TYLCV) on tomato and *Cassava brown streak virus* (CBSV) on cassava [74], causing serious economic losses to crop growers worldwide and threatening food security in Africa and elsewhere [75]. A new type of whitefly (*B. tabaci*, biotype B) has increased dramatically in both density and geographic distribution, arguably as a result of global warming. A project initiated in 2013 is aimed at developing RNA interference (RNAi) technology using target genes from transcriptome, small RNA, and genome sequence assemblies. The *B. tabaci* genome size was recently estimated to be 690 Mb using a combination of k-mer and flow cytometry analyses [76], which was nearly 300 Mb smaller than previously reported [77]. In a proof-of-concept study, the whitefly Vacuolar ATPase-A gene was silenced using RNAi technology using hydroponic tomato leaf-mediated delivery, and RNA interference of the target gene expression was verified using sequence-specific real-time quantitative PCR (qPCR). Efforts to develop a whitefly cell line are underway in consultation with an ARS scientist at the U.S. Horticultural Research Laboratory in Fort Pierce, FL [78]. Whitefly cell lines would provide a valuable tool not only for screening RNAi targets, but also for understanding the mechanisms regarding virus invasion, replication and secretion in whitefly. An effective control strategy to manage these emerging whitefly-transmitted viruses is currently limited. RNAi technology offers a great potential to control whiteflies and the viruses they transmit [79], and work is ongoing to develop these genomic tools into real world applications.

2.4. Improving the protection of stored grain products

Arthropod pests damage millions of bushels of stored grain each year that was valued at US$64 billion in 2008, and inflict up to 9% post-harvest losses [80]. Larvae of the yellow mealworm, *Tenebrio molitor*, and the red flower beetle, *Tribolium castaneum*, have highly compartmentalized guts that primarily use cysteine peptidases in the acidic anterior midgut for the early stages of protein digestion. A high-throughput NGS approach was used to obtain a gut transcriptome, from which C1 papain family cysteine peptidases were characterized [81]. Gene annotation predicted that 25 *T. castaneum* genes and one questionable pseudogene encoded cysteine peptidases (11 cathepsin L or L-like, 11 cathepsin B or B-like, and one each of cathepsin F, K and O), and transcripts of greatest quantity were from two cathepsin L genes on chromosome 10 (LOC659441 and LOC659502). Cathepsin B expression was mainly from genes on chromosome 3 (LOC663145 and LOC663117), whereas cathepsins F, K and O transcripts were expressed at lower levels or were undetectable in the larval gut. Analogously, 29 *T. molitor* cysteine peptidase genes were predicted (14 cathepsin L or L-like, 13 cathepsin B or B-like, and one each of cathepsin O and F). One cathepsin L and one cathepsin B orthologous to those highly expressed in *T. castaneum* were also highly expressed in *T. molitor*. Peptidases lacking conserved active site residues were identified in both insects, and sequence analysis of orthologs indicated that changes in these residues occurred prior to evolutionary divergence. Cysteine peptidase substrate binding region sequences had a high degree of variability, consistent with the ability of these enzymes to degrade a variety of cereal seed storage proteins and inhibitors. Predicted cathepsin B peptidases from both insects included some with a shortened occluding loop without active site residues in the middle, apparently lacking exopeptidase activity and unique to tenebrionid insects. Docking of specific substrates with models of *T. molitor* cysteine peptidases indicated that some insect cathepsin B and L bind substrates with affinities similar to human cathepsin L, while others do not, and have presumably different substrate specificity. These studies have refined our model of protein digestion in the larval gut of tenebrionid insects,

and suggest genes that may be targeted by inhibitors or RNAi for the control of cereal pests in storage areas.

3. Management and control of arthropod pests affecting livestock

Arthropod pests of veterinary importance remain a threat to the health of livestock herds in the United States and contribute to global food insecurity because they impact animal agriculture productivity directly through their parasitic habits and indirectly, in specific cases, due to the disease-causing agents they transmit, which in some instances can also affect humans [82, 83]. The ARS conducts basic and applied research to deliver science-based technological solutions to the problems producers have with veterinary pests like ticks and biting flies. In several cases around the world, the emergence and re-emergence of problems with the control, eradication, or re-eradication of veterinary pests are driven by forces related to global change [84]. Veterinary pests can be of high-consequence when they become invasive. The ARS strategic plan contemplates the prevention and control of pests and animal diseases that pose a threat to agriculture and public health [21, 85].

Basic and applied research efforts at several ARS laboratories aim to develop and transfer tools to the agricultural community, commercial partners, and government agencies to control or eradicate domestic and exotic diseases and pests that affect animal and human health. Some of the strategies to manage pest populations include genomic applications that aim to restrict cattle fever ticks to a defined quarantine zone, maintain the eradicated status of the New World screwworm in North and Central America, and to develop sustainable control methods for stable and horn fly species. These cumulative efforts provided the impetus to establish a virtual Veterinary Pest Genomics Center (VPGC) as part of the overall strategy to improve human and food-animal health through the development of effective and safe pest control methods.

The USDA-ARS VPGC leverages big data solutions to evaluate risk and mitigate the impact of invasive veterinary pests, and has used key biological resources, NGS technology, and state-of-the-art bioinformatics approaches to sequence and annotate the genomes, transcriptomes, proteomes and metagenomes from arthropod pests of veterinary importance [86]. The VPGC has developed and used molecular tools for population genomics studies of veterinary pests in indigenous and invasive ranges in order to understand the role of evolutionary forces in shaping phenotypic variation [87, 88]. The VPCG also applied biogeographic, spatial and temporal analyses to quantify and predict economically important or potential veterinary pest distributional changes, and integrated these analyses with genetic studies of rapid evolution and adaptation of pests to new or changing environments [89, 90]. Furthermore, the VPCG conducted feasibility studies to evaluate advanced computing hardware and software systems for their ability to store and analyze large data sets on veterinary pests, with the capacity to integrate results from longitudinal environmental assessments [91, 92].

Examples of how VPGC is helping unravel and mine the genome of high-consequence livestock pests appear below. These mutually productive collaborations are enabling synergies among and between ARS laboratories and research partners to develop and utilize genomics-based solutions. Research partnerships facilitated by VPGC provide the opportunity to translate research into transformative technologies that can be integrated to mitigate the economic impact of veterinary pests, which will promote precision agriculture to enhance animal protection and production.

3.1. Genomics-based target discovery for a vaccine against cattle tick

A major focus of molecular and functional genomics research at the USDA-ARS Knipling-Bushland U.S. Livestock Insects Research Laboratory (KBUSLIRL) is the southern cattle fever tick, *Rhipicephalus* (*Boophilus*) *microplus*, which is regarded as the most economically important external parasite of cattle worldwide [93]. This tick is the vector of the apicomplexan protozoa *Babesia bigemina* and the more virulent *B. bovis*, which cause bovine babesiosis or cattle tick fever, and *Anaplasma marginale* that causes anaplasmosis [83]. The annual losses to the U.S. cattle industry

attributable to *Rhipicephalus* ticks were estimated in 1906 to be $130,500,000 (equivalent to over $3 billion in today's dollars). The USDA-APHIS Cattle Fever Tick Eradication Program (CFTEP) successfully eliminated this tick from the United States by 1943. However, pathogen-infected cattle fever ticks (CFT), *R. microplus* and *R. annulatus*, are established in foreign nations. The CFTEP maintains a 500-mile "buffer zone" along the southern US border with mandatory acaricide treatment of livestock prior to importation into the United States, wherein the annual costs for this program exceeds $4 million. In recent years, *R. microplus* has repopulated the southern counties of Texas despite the continuing efforts by the CFTEP. Only a few classes of acaricides are approved for the treatment of *R. microplus* on cattle, and resistance to commercially available acaricides has spread extensively across the cattle tick population in tropical and subtropical regions of the world [94], which indicates that novel control technologies will likely greatly benefit the cattle industry [95]. ARS researchers at KBUSLIRL identified candidate tick antigens and developed a novel anti-cattle tick vaccine that could be used alone or in combination with synthetic acaricides [96]. Trials using vaccines produced from recombinant antigens show 63-76% efficacy against *R. microplus* in cattle pen trials in Brazil. Two of the vaccine antigens discovered have been patented and are currently being evaluated for their potential development as tick control agents. The reverse vaccinology approach has been applied to discover other candidate antigens by mining the genome of *R. microplus* [97, 98]. This approach combines genome and transcriptome sequencing with bioinformatics and molecular biology tools such as high-throughput proteomics, microarrays, and RNAi. The genome of *R. microplus* is large (2.5 times the size of the human genome) and composed of ~70% repetitive DNA [99]. A combined Illumina- and PacBio-based approach achieved 10 times the sequence coverage of the *R. microplus* genome, which enabled the genome to be assembled despite the high number of repetitive sequences. Concomitantly, transcriptome studies have identified transcripts critical to tick development, feeding and pathogen transmission, including those considered priority vaccine antigen candidates [92]. This project demonstrates how discovery-based genomics of a veterinary pest yields direct outputs with potentially large beneficial impacts upon agriculture.

3.2. Developing transgenic, male-only strains for screwworm eradication

The USDA-ARS, Screwworm Research Unit (SRU), Kerville, TX is working in collaboration with North Carolina State University (NCSU) to develop sexing screwworm strains. The eradication of screwworms from mainland North America using the SIT was an unprecedented achievement saving livestock producers at least $1.6 billion annually [100]. Reinfestation currently is prevented by maintaining a permanent barrier at the Panama-Colombia border, and the ARS Screwworm Research Unit investigators support these efforts by rearing and studying the ecology and genetics of screwworms. Male-only insect strains have been successfully developed, using sex-autosome translocations, and have great potential for lowering costs and improving the quality of released sterile males [101]. However, problems with stability of these strains occur during mass rearing that impede the implementation of screwworm eradication [102]. The development of transgenic techniques for several insect species other than *Drosophila*, including screwworms, has demonstrated that the genetic engineering of repressible, lethal, female-specific genetic systems is possible for screwworms [103]. Efficient implementation of transgenic techniques will require reasonable understanding of genetic linkage, sex-determination and embryonic development in screwworms to aid in determining that resultant transgenic strains are stable.

Five genetic sexing screwworm strains have been developed that express a single-component, late-acting, tetracycline repressible female lethal genetic system, and are currently being examined to determine their utility in the eradication program. SRU and NCSU researchers also have measured various fitness parameters that influence production including biological yield, egg hatch and longevity, and several lines are comparable with the parental strain necessary to gain regulatory approval. More recently, development of a two-component, early-acting, tetracycline repressible, female lethal genetic system has been initiated. In this system,

early expression of *tTA* in the embryo leads to activation of expression of a cell death gene, which leads to death of the embryo. Only females die as the cell death gene contains the sex-specifically spliced NWS *tra* intron. Success and implementation of this project would lead to significant savings in diet costs (up to 50%, ≥$500,000/year at current rearing levels) and/or increase in the production capacity.

4. Management and control of invasive arthropods

Functioning ecosystems are comprised of balanced trophic interactions among indigenous species within the local environment. Arthropods fulfill a myriad of ecosystem services, from predators, prey and herbivores to detritivores (see Introduction). Inadvertent introduction of exotic arthropod species into non-native areas can have dire consequences on species diversity and community structure at all levels within newly inhabited environments [104, 105], and is one potential factor that may lead to extinction of native species [106]. The impact of arthropod invasions on agricultural and forestry production can be equally catastrophic. The historical accidental introduction of the European corn borer, *O. nubilalis* into the United States in the early 1900s serves as one such example and is discussed above. Introduction of this species has led to sustained economic losses to naive and inadequately protected crops as this insect has spread across the major corn production areas of the Midwest [107]. Similarly, the soybean aphid, *Aphis glycines* which is native to Asia, was discovered in the Midwestern United States in 2000 [108]. After spreading across soybean production areas, *A. glycines* has been linked to severe reductions in plant health and yield [109], and vectoring of soybean dwarf and mosaic viruses [110, 111]. Forests and forestry production currently are threatened by the introduction of more than 50 known exotic wood boring beetles in the United States [112]. These beetles damage live trees by direct feeding and by spreading phytopathogenic fungi [113], and in some instances has resulted in tree death and damage across very large forested areas. This includes the Asian longhorned beetle, *Anoplophora glabripennis* that damages several deciduous genera within the United States [114].

Several additional examples of non-native arthropods regarded as particularly detrimental to plant and animal health if introduced into the United States or spread further into new geographic ranges include the citrus brown mite, Mediterranean fruit fly, cotton bollworm, *Targionia* scales, New and Old World screwworms, louse fly, sheep scab mite, cattle ticks, bont ticks, brown ear tick, sea lice, and European green crab, which are projected to cause severe economic impacts (Table 3). Conservative estimates of the overall cost associated with invasive pest arthropods in the United States exceed US$120 billion per year [115] and are greater than US$1 trillion worldwide [2]. Protecting plant and animal health, and enhancing biosecurity and quarantine measures remain critical for safeguarding the U.S. food supply. Invasive species management entails monitoring (detection and tracking) followed by mitigation or eradication as a means of control. USDA Animal Plant Health Inspection Service (USDA-APHIS) inspects imports at over 330 airports and harbors for the presence of foreign plant, animal or microbial materials. This first line of defense is augmented by state and local detection guided by expertise at state extension programs, and agriculture and natural resources departments that assist landowners in confirmation of putative invasive species. Potential for an introduced species to become invasive can be modeled by comparison of environmental conditions between introduced and native ranges [116], comparison of traits shared with previous invasive arthropods, or by the risk assessment of a resulting invasion [117].

In response to the increasing influx of invasive species into the United States, ARS scientists have made strides in understanding the biology of non-native species within the geographic regions they have been introduced. For the one example below, genomic tools inclusive of a whole genome sequence and gene expression studies have provided major insights into how the imported fire ant has successfully colonized areas of the southern United States. Not to be overlooked, but both the cattle tick and screwworm in the case studies highlighted above are also invasive arthropods which might indicate the continual importance of invasive arthropods.

Table 3. Economic impact of selected invasive arthropods in the United States.

If the New World screwworm were eradicated in South America, which would alleviate concerns for reintroduction into the United States, an estimated US$3.5 billion could be saved each year [230].
Previous estimates converted to today's currency rate indicate that the livestock industry realizes annual savings of at least US$3 billion dollars since the U.S. was declared free of the deadly disease bovine babesiosis and its cattle fever tick vectors; however, the invasive cattle fever ticks are established in Mexico and remain a threat to animal agriculture in the U.S. [84].
A recently introduced long-horned beetle severely threatens hard wood forests and several species of trees in urban and native landscapes. Efforts to eradicate this beetle already exceed US$500 million; the total estimated economic loss if allowed to spread is US$700 billion [231].
Sea lice, which are ectoparasitic crustaceans, are the most damaging parasite to the salmon farming industry, and the annual economic costs of sea lice control is US$480 million [232].
European green crab, which was introduced as a food source, destroys commercial shellfish beds and preys on large numbers of native oysters and crabs. The annual estimated economic impact of this crab is US$44 million [233].

4.1. Population and functional genomics of invasive fire ants

One introduced pest of significant importance in the United States is the invasive fire ant *Solenopsis invicta* that was inadvertently introduced from South America some 75 years ago [118-121]. It has subsequently spread throughout the southern US [122, 123] and more recently to several western states (e.g., New Mexico, California), as well as the Caribbean, Australia, China, and Taiwan [124-129]. Concerns about the serious negative economic and ecological impacts of *S. invicta* have led to the development of many different control methods that target individual nests (e.g., contact insecticides) or are intended to suppress the colonies inhabiting larger areas (e.g., baits containing poisons, growth regulators, or reproductive inhibitors) [130-132]. Because these methods generally have failed to halt the continued spread and population increase of this invasive species, alternative approaches to population management, such as those based on biological control by natural enemies imported from the native range, are being developed in attempts to suppress introduced populations over the vast areas they now occupy [118-121, 133-138]. Clearly, however, there is an urgent need to develop additional effective and safe alternative management techniques for this pest ant that can complement existing control methods. Development of these alternative management techniques requires intimate knowledge of factors such as the immune function, reproductive physiology and social behavior of fire ants, and the underlying genetic determinants of these biological features, because these represent attractive targets for disruption and manipulation culminating in population suppression.

Genomics research conducted at the USDA-ARS Imported Fire Ant Research Unit (IFARU) and other institutions produced the first draft of the *S. invicta* genome [139] and subsequently developed new functional genomics resources including a large panel of molecular genetic markers, a cDNA-based oligonucleotide microarray, a genetic linkage map, and application of targeted gene knockdown using RNAi [140-144]. These tools are being used to investigate novel control strategies and understand the fire ant natural history, physiology, genetics, chemical ecology and the molecular basis of their susceptibility to control agents. For example, IFARU researchers detected variation at a large number of genetic marker loci and identified the native source population(s) of the *S. invicta* colonizing the southern United States [145]. Previous data suggested the existence of high levels of genetic differentiation among different geographic *S. invicta* populations occupying the vast native range. Such strong differentiation is consistent with the hypothesis that natural enemies of *S. invicta* are locally adapted to their hosts. Thus, the success of natural enemies from a given locale in attacking *S. invicta* likely depends

on the geographic origin of the host ants. If so, efforts to pinpoint the source population become highly relevant to the identification of effective biological control agents of introduced *S. invicta*. Subsequent studies reconstructed the global invasion history of *S. invicta* into the US, which predicted at least nine separate introductions and the main southern United States population likely originated from all but one of these introductions [146]. This study was the first to employ such a large number of genetic markers in a non-model organism and is used as a guide for similar studies in other introduced insects.

Recently, the genomic region responsible for two divergent forms of colony social organization in fire ants was identified using a combination of RAD-seq, genome re-sequencing and BAC-FISH [143]. This genomic region was shown to be part of a pair of heteromorphic chromosomes having many of the key properties of sex chromosomes. The non-recombining region encodes most genes that show differences in expression between individuals from these two social forms. These findings highlight how genomic rearrangements can maintain divergent adaptive social phenotypes involving many genes acting in concert by locally limiting recombination. Currently, ARS researchers are using RAD-seq to develop SNP markers for additional population genomic studies in fire ants. Analogously, bioinformatic analyses of genome and transcriptome sequence data led to the identification of three novel viruses that infect fire ants [147], of which two are similar (but clearly unique) to RNA viruses (SINV1-3) and a single-stranded DNA virus (densovirus) previously described from fire ants. Laboratory studies of these viruses are currently underway to better understand their phenotypic effects and potential for use as natural biopesticides.

5. Management and improvement of beneficial arthropod health

Arthropod effects on agriculture are not always detrimental. Arthropods also significantly impact the earth's ecosystem via carbon cycling and can improve agriculture by filling important ecological roles and performing ecosystem services. Numerous beneficial insects exist and are crucial to sustainable agricultural production. For example,

one-third of row-crop plant species as well as most orchard and horticultural crops depend on insect pollination for pollen dispersal and subsequent fruit production. The honey bee, *Apis mellifera*, alone pollinates US$15-20 billion worth of crops annually in the United States and the annual estimated value of all ecological services provided by non-domesticated insects is at least US$57 billion [148]. Worldwide, pollination by *A. mellifera* is estimated to increase annual crop production by US$60 billion through more bountiful seed, fruit, nut and plant harvests [149]. Additionally, numerous products directly derived from bees including honey, wax, and propolis, comprise a billion-dollar cash crop for farmers in both developing and developed nations. Understanding bee behavior, ecological function, and health has become increasingly important given the emergence of colony collapse disorder (CCD) in 2006 [150, 151]. Hives affected by CCD show, among other symptoms, marked decreases in the number of worker bees and a puzzling absence of dead bees within and around the hive [152].

Predation and parasitism by natural enemies reduce pest arthropod populations, such that enhancing the efficacy of biological control may improve the sustainability of production practices by decreasing the sole reliance on pesticides within IPM approaches. Benefits to producers accrued from biological control research have far outweighed the initial investment costs [153]. For example, a near US$1 million annual research investment in alfalfa weevil biological control yields a net savings in excess of US$48 million. Overall, biological control measures conservatively provide US$2 billion per year in savings to growers across all commodities, but undocumented control in natural systems may likely exceed this value when broadcast pesticide applications are reduced.

Enhancing the activities of beneficial insects is not only in the general public interest, as in the case of the sentinel species such as the honey bee and the monarch butterfly, but also for protecting crucial biological and ecological roles these insects play in enhancing agricultural production. Specifically, the roles of many insects are fundamental for several ecological functions including 1) pollination of flowers in fruit and

vegetable crops and 2) natural suppression of insect populations that are pests in plant and livestock production (many discussed above). Genomics research at ARS briefly presented below has made direct contributions to safeguarding honey bee health and enhancing the efficacy of natural enemy populations.

5.1. Honey bee health and management

USDA-ARS research on honey bee health is carried out at several locations including the Bee Research Laboratory (BRL, Beltsville, MD), the Honey Bee Genetics and Breeding Laboratory (Baton Rouge, LA) and the Cal Hayden Bee Research Center (Tucson, AZ). Several avenues of research at these locations conduct genomic analyses to understand honey bee nutrition and the impacts of microbiota in the beehive on bee nutrition and health. Several other ARS laboratories (i.e., at Fargo, ND, Ft. Pierce, FL, Gainesville, FL and Stoneville, MS) also have active honey bee research programs targeting honey bee stress and parasite reduction. Finally, research on native bee pollinators is carried out at the USDA-ARS Pollinating Insect Biology, Management and Systematics Research Laboratory in Logan, UT.

Scientists at several of these ARS research locations have been instrumental in efforts to unravel the potential causes of CCD. ARS scientists helped lead efforts to sequence the honey bee genome with the aim to improve honey bee breeding and management [154]. This effort led to a vast improvement in publically available resources describing honey bee genes [155], sequence variants [156], traits associated with changes in genome structure [157], and gene expression [158]. For example, honey bee genes and pathways involved in immune response to disease agents were identified [159] and used to generate markers for breeding disease resistance traits [160]. Honey bees also were shown to have a diminished capacity to produce normal detoxification enzymes found in other insects [161], which may partially explain their frequently observed hypersensitivity to pesticides [162]. Additional global analyses of these detoxification responses have generated biomarkers for bee declines [163], and identification of pesticide classes that can be detoxified effectively and thus

more compatible with efforts to sustain bee health [164], as well as provided insight into synergistic adverse effects of pesticides and pathogens [165]. Early on, ARS research at BRL was instrumental in identification of a differentially-expressed gene common among CCD affected bees, which led to the development of a biomarker for diagnosing CCD [163]. Furthermore, a metagenomics study comparing normal and CCD affected hives showed a strong association with the Israeli acute paralysis virus in the latter [152].

These genomic tools have led to an improved understanding of honey bee biology and have enabled applications for better beekeeping. For example, biomarkers indicative of honey bee queen production and physiology were identified, leading to diagnostic tools for identifying healthy queens [166]. Gene expression was shown to be different between the brains of nurse vs. forager bees, which provided insight into bee behavior and the caste system of social insects [167]. Identification of an immense array of olfactory proteins provides a tool for managing bee behavior and development, and supports theoretical predictions that social insects communicate by smell and use this sense more strongly than the sense of taste [168]. Honey bees were found to have a fully functional methylation system that is involved in the regulation of gene expression [169], which was later linked to a series of changes during caste differentiation between the queen and worker honey bees [170, 171]. Using the genome assembly as a template, each major race of honey bees has been re-sequenced, and comparisons among these genomes has provided insight into bee behavior [172], diversity [173] and aggressive africanization [174].

5.2. Phylogenomics and genetics of biological control introductions

The USDA-ARS Beneficial Insect Introductions Research Unit (BIIRU), Newark, DE, investigates the phylogenomics and genetic architecture of parasitoid host specificity to support the ecologically safe and effective control of invasive pest arthropods. Invasions by exotic species that become pests are an increasing problem for agriculture. Biological control by introduction of natural enemies has proven effective at reducing the abundance and

impact of such pests, and in principle provides a safe, cost-effective alternative to widespread application of insecticides. Host specificity testing is designed to ensure that introduced organisms intended to control pest insects do not impact native non-target species [175, 176]. However, introduced natural enemies may evolve after introduction [177], and if host specificity shifts rapidly, traditional screening may not guarantee the safety of biological control introductions. Furthermore, invasive species populations can quickly reach high densities, which may lead to behavioral or evolutionary shifts by native natural enemies, thus arguments have been made that the control of the invading pest might be achieved without the introduction of non-native species [178, 179]. Parasitoid Hymenoptera are natural enemies of several pest insects, and although much is known about their foraging and host selection behavior [175, 180, 181], the evolutionary stability of parasitoid host specificity has received scant attention [182, 183]. The working hypothesis is that parasitoid host range is determined by epistatic gene interactions (i.e. non-additive effects among loci) and evolutionary changes in host specificity are unlikely. Little is known about the genetic architecture of host specificity for any insect or the evolutionary mechanisms involved in shifts in host specificity [182-185]. Predicting potential shifts in host specificity depends on the knowledge of the genes that are involved and their potential interactions [186]. The genus *Aphelinus* (Hymenoptera: Aphelinidae) comprises 90 recognized species [187, 188] and are internal parasitoids of aphids. USDA-ARS, BIIRU led efforts that used DNA sequence divergence and prediction of reproductive isolation to identify six cryptic species in the *varipes* complex of *Aphelinus*, which provides a framework for mating behavior and host specificity studies [189]. Similarly, USDA-ARS, BIIRU was instrumental in defining the *mali* complex for *Aphelinus* [188].

The Russian wheat aphid, *Diuraphis noxia* Kurdjumov (Hemiptera: Aphididae) is a major pest of small grains in the United States [190] that was first detected in 1986 [191], and is the target for the biological control agent *Aphelinus atriplicis* [192, 193]. Despite introduction of resistant wheat varieties in 1996, *D. noxia* remains a pest due to host plant adaption [194], but the European,

Aphelinus hordei, is a promising candidate for introduction against *D. noxia*. Analogously, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native of Asia first found in North America in 2000. In Asia, *A. glycines* occasionally reaches damaging levels, but in the United States, it has become a major pest of soybean, infesting 42 million acres and costing an estimated US$1 billion in 2003 alone [195]. The USDA-ARS, BIIRU recently discovered and described three new species in the *mali* complex, *Aphelinus coreae*, *Aphelinus glycinis*, and *Aphelinus rhamni*, in collections made from China and Korea, and despite close in morphology, are reproductively isolated, and show differences in DNA sequence and in host specificity [188]. These new species have narrow host ranges, and *A. glycinis* and *A. rhamni* are being introduced against soybean aphid.

USDA-ARS, BIIRU mapped parasitism of aphid species onto a molecular phylogeny of *Aphelinus* [189], which revealed two distinct host range patterns: 1) inheritance from a common ancestor, versus 2) indication of speciation driven by host shifts. Genome and transcriptome sequence assemblies have been completed for 10 species of *Aphelinus* in four species complexes (Hopper *et al.*, unpublished data). These draft assemblies had 98-100% of 248 conserved genes in the core eukaryotic genes [196], indicating assemblies are likely representative of full gene complements. Annotation using AUGUSTUS [197] predicted 25k-36k putative coding sequences, of which 20k-27k (70-80%) had hits in the GenBank RefSeq database using BLASTP [198], and 25k-35k (90-100%) of which had RNA transcripts. Because host specificity in *Aphelinus* species results primarily from host acceptance, female chemosensory genes are prime candidates for determining host specificity. Among the BLASTP hits, 33 to 58 odorant receptor proteins, 4 to 12 odorant binding proteins, and 15 to 31 gustatory receptor proteins were predicted within assemblies. Roles of these genes in determining parasitoid host range are being investigated using QTL mapping approaches.

6. The future of agriculture-focused applied arthropod genomics

Although agriculture has benefitted from technological advances that spawned dramatic

increases in production over the past several decades, the world's growing population will continue to place additional demands on this economic sector. In addition to challenges of increasing domestic and export market demands, calls for environmental stewardship by consumers are expected to increase as well. Arthropod damage and disease transmission within agroecosystems accounts for significant yearly losses in virtually all aspects of agricultural production (see Introduction), and better management of arthropod pest species through translational genomics research is likely to be a crucial component in developing strategies to protect food, fiber and biofuel production. Additionally, protecting beneficial arthropods responsible for pollination and biological control of pest species remains an important component of sustainable agricultural production and environmental stewardship.

NGS technologies use pyrosequencing reactions, sequence-by-synthesis (SBS), sequencing by oligo ligation and detection (SOLiD) [199], ion semiconductor sequencing [200] and single molecule sequencing methods [201], among others, to acquire large amounts of nucleotide data. These advances have fundamentally changed the scope of scientific inquiry, from the study of one or a few genes towards a systems approach that accounts for variation across an entire genome or transcriptome. These platforms and applications alleviate expenses and technological hurdles previously associated with genome-scale studies, which had effectively restricted genomics to a select few model organisms. A host of reviews can be found discussing potential outcomes and applications in functional genomics [202], population genomics [203], ecological genomics [204], and adaptation studies [205], and hence are not addressed further here. Application of these genomic principles to non-model arthropod species of agricultural significance holds enormous promise for studies spanning the breadth of ARS arthropod research, and was shown by way of the ARS-led genome assembly for the coffee berry borer, *Hypothenemus hampei* [206]. Fundamental studies using genomics, transcriptomics, and reverse genomic approaches will lead to the elucidation of arthropod biochemical, cellular and physiological processes (e.g., growth and reproduction, stress response, metabolism and adaptation) via the identification of genes, regulatory elements and genetic variants. Population- and ecological-scaled studies applying genomics can account for changes in genetic structure of key organism populations (pests, predators, parasitoids and beneficial arthropods) in agroecosystems on a genome-wide scale and have potential for use in developing effective IPM strategies, understanding adaptation to management practices, and protecting the health of beneficial pollinators. This biological systems approach requires fundamental knowledge of pest interactions with hosts, predators, parasitoids and pathogens, and how management practices impact arthropod communities [207]. Ecological genomics is becoming an increasingly important concept for understanding genome structure and function within the landscape [208-210]. More broadly, the genomics of insect-crop plant and insect-animal interactions may to be used to bolster host resistance through increasing our understanding of how evolutionary histories and environmental interactions affect short-term response and long-term adaptive changes. Conversely, looking more fine-grained within arthropods has revealed the importance of symbiotic bacteria in pest survival on agriculturally important plants. For instance, the symbiotic bacteria *Buchnera* provide key biosynthetic products necessary for the survival of aphid species feeding on nutrient-poor plant phloem [211]. Recent studies conducted at ARS demonstrated the importance of gut microbiota for the detoxification of caffeine by the coffee berry borer, *H. hampei*, the most damaging pest of coffee in Hawaii [212]. The investigation of bacterial-insect interactions often is investigated through metagenomics studies [213], and the range of co-evolutionary relationships found therein continues to be discovered and may represent a novel resource for technological applications [214].

Genome and transcriptome sequencing projects generate 'reference' sequences and associated functional gene annotations that are resources for other downstream analyses by the larger research community, which further facilitate genomics research within and across arthropod species. Even if a reference genome is not available for the particular species of interest, synteny and

co-linearity among genomes of closely related species can be used to inform annotation of novel sequence (via inference of orthologous relationships [215]) and may assist with reference-based assemblies. Keys to the effective re-use of reference genome sequences include ease of accessibility and proper curation and maintenance. Curation of gene models is important to ensure the accuracy of computational predictions from nearest model species, but is not guaranteed to be correct without empirical testing [216]. Providing access to curated genome data requires dedicated storage infrastructure and clear data management goals. Several genome portals that perform some or all of these functions already exist for certain arthropods (*cf.* VectorBase [217]; Hymenoptera Genome Database [218]; and many others). The National Agricultural Library has implemented the i5k Workspace@NAL to provide access to "orphaned" arthropod genome sequences and annotations, and as a platform for community-based genome curation and maintenance (https://i5k.nal.usda.gov/; [219]) including for many arthropods that are important agricultural pests. Portals such as the i5k Workspace assist in the dissemination,

visualization and curation of genome data by facilitating access, and providing unified platforms for genome sequence curation and management. Given the ever increasing output of NGS platforms and relative ease by which genome-scale data can be generated, data management may become an increasingly important part of genomic research.

Selected examples of current ARS translational research were outlined previously in the article, and whole genome and transcriptome data from arthropods coupled with detailed population and functional genomics studies will provide enormous benefits to crop and animal health and production, with possible outcomes not limited to additional outcomes listed in Table 4. The ARS mission is to drive translational research by applying fundamental baseline research to develop solutions to current agricultural problems. Overall, the returns from taxpayer investment in agricultural research are high [220], which help to keep the costs of agricultural production competitive in world markets [221] and to maintain a safe and stable food supply for consumers. Since the advent of genome-scale sequencing technologies and analyses, genomics has been shifted from prospect and promise to

Table 4. Potential future applications of arthropod genomics research.

Insect pests of plants and animals (including disease vectors)
Identify genomic basis of pesticide resistance; discover novel ways to circumvent the onset of resistance and new targets for pest insect control.
Disrupt relationships between arthropods and pathogens to prevent pathogen transmission and diseases.
Identify new antigens for vaccine development applied to arthropod vector control.
Enhance sterile insect technique (SIT) programs by incorporating new and complementary methods in area-wide approaches to control pests and to eradicate local populations of pests.
Determine the origins and population dynamics of invasive pest insects to aid in tracking and eradication.
Identify new insect vector target genes leading to improved strategies for disease control.
Identify molecular underpinnings of key adaptations of invasive insects, which in turn can serve as targets for population control.
Beneficial insects and pollinators
Improve health and disease resistance of beneficial arthropods and pollinators.
Understanding of the biological and genetic potential of insect nutrition, reproduction and immunity.
Identify microbial communities of arthropods that enhance or reduce susceptibility to insect pathogens.
Discover and develop novel biocatalysts from insects and associated microbes for biorefinery applications.

realities in the agricultural sciences. Arthropod genomics research at ARS has and will continue to contribute significant improvements for agricultural practices, and undoubtedly directly benefit farmers, the agricultural industry and consumers.

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CONFLICT OF INTEREST STATEMENT

Authors claim no conflicts of interest.

ABBREVIATIONS

CCD	:	Colony collapse disorder
BAC	:	Bacterial artificial chromosome
FISH	:	Fluorescent *in situ* hybridization
i5K	:	5000 arthropod genomes initiative
IPM	:	Integrated pest management
IRM	:	Insect resistance management
GBS	:	Genotyping by sequencing
NGS	:	Next generation sequencing
PCR	:	Polymerase chain reaction
SIT	:	Sterile insect technique
RAD-seq	:	Restriction enzyme associated DNA markers
RNAi	:	RNA interference
RNA-seq	:	RNA sequencing
QTL	:	quantitative trait locus
USDA-APHIS	:	United States Department of Agriculture, Animal & Plant Health Inspection Service
USDA-ARS	:	United States Department of Agriculture, Agricultural Research Service

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