Genetic flexibility in the green peach aphid: Defensive polyphenism in response to parasitoid pressure and the assembly of a new draft genome

by

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Abstract

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), poses a serious threat to a wide variety of both greenhouse and field crops. In greenhouses, biological control agents are commonly used to combat *M. persicae* infestations.

In order to better understand how *M. persicae* adapts to parasitoid pressure, I designed a multi-generational experiment using a classical experimental evolution framework, where single genetic lines of *M. persicae* were exposed to the parasitoid *Aphidius colemani* over multiple, consecutive generations. The results of this experiment show no evidence that *M. persicae* adapts to parasitoid pressure over time, and hint at the importance of aphid colony density in transgenerational stress responses.

In recent years, genomic analysis has become an increasingly useful tool for investigating aphid polyphenism. Using PacBio long-reads, I generated a new draft genome assembly for *M. persicae* that I hope will contribute to aphid genomic studies in the future.

Keywords: *Myzus persicae*, polyphenism, experimental evolution, biological control, draft genome.

Dedication

I dedicate this thesis to my parents, Yuval and Yolande, who shared with me their love for learning and taught me to never stop asking questions.

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Glossary

Apomixis	A form of parthenogenesis where diploid ova are produced through simple mitosis.
Bt crop	Crop plants that have been genetically modified to produce the one or several Bt toxins, which are natural products of the entomopathogenic bacteria *Bacillus thriunugensis*.
Conidia	Asexual fungal spores.
Contig	A single, contiguous stretch of genomic DNA which has been assembled from sequencing reads.
Cornicles	Small tube-like pores at the posterior end of the aphid abdomen. Used to deliver aphid alarm pheromone.
Haplotig	Two contigs which map to homologous, non-identical regions on sister chromatids.
Koinobiont	A parasitoid whose host continues to grow and feed after parasitization
Thyletokous Parthenogenesis	An asexual mode of reproduction where embryos develop from unfertilized ova
Polymorphism	Where multiple phenotypes may be produced within a genetic line based on genetic differences between individuals.
Polyphenism	Where multiple phenotypes may be produced from a single genotype based on environmental conditions.
Transcriptome	The complete set of mRNA transcripts produced by an organism at a given point in time.

Chapter 1. Insect resistance management and the green peach aphid, *Myzus persicae* (Sulzer)

The management of insect pests in agriculture is a massive global industry. In 2012, global expenditures on insecticides topped US$56 billion (Atwood and Paisley-Jones 2017). In that same year, the use of insect-resistant genetically modified maize and cotton increased global farm income by US$12 billion (Brookes and Barfoot 2014). Worldwide, the management of animal pests results in a ~40% annual reduction of monetary yield losses in field crops (Oerke 2006). Despite this investment in crop protection, crop losses caused by insect pests continue to have a significant economic and human impact; the diamondback moth *Plutella xylostella* alone is estimated to cause up to US$5 billion annually in global crop losses (Zalucki et al. 2012). One factor contributing to the continued threat of insect pests is the development of resistance to control techniques.

Effective Integrated Pest Management (IPM) should account for the likelihood that a given pest will adapt to management tactics over time. A successful example of this is high dose/refuge cropping in Bt crops. In high dose/refuge cropping, two crops are planted adjacent to one another – a “high dose” crop which produces a lethal concentration of Bt toxin, and a “refuge” crop which produces no toxin. This strategy assumes that (*i*) Bt-resistance is a recessive genetic trait, and that (*ii*) Bt-resistant homozygous individuals emerging from the high dose crop will mate with equal frequency with other resistant individuals and with non-resistant individuals emerging from the refuge crop. Since offspring of this mating would be heterozygous at the resistance locus, and are thus just as susceptible to the Bt toxin as their non-resistant parents, this strategy prevents Bt-resistance genes from becoming fixed in pest populations (Bates et al. 2005; Camargo et al. 2018). Preventative IPM tactics are typically described under the umbrella of preventative Insect Resistance Management (IRM) (Onstad 2014). Insect biological control is one area lacking in IRM.

Biological control, or biocontrol, is the practice of managing pests using living organisms. Biocontrol of insects is typically accomplished through the application of predators, parasitoids, or entomopathogenic fungi, and is often employed in cases

where insect pests have developed resistances to insecticides (van Lenteren 2000; Powell and Pell 2007; Bale et al. 2008). Development of resistance to biocontrol agents by insect pests is rarely reported; thus far, there has been only one report of an insect pest developing resistance to a parasitoid wasp (Tomasetto et al. 2017). The true rate of development of resistance is likely underestimated, possibly due to a lack of post-release monitoring in many IPM programmes (Barratt et al. 2006; Van Driesche and Hoddle 2016). It is a truism in biology that “life escapes all barriers” (Crichton 1990), and it is my belief that IPM researchers and professionals should be cognizant of this when designing protocols.

One important factor to consider when developing IRM protocols is the reproductive strategy of the pests in question. In Bt-crops, high-dose/refuge cropping tactics preserve a population of susceptible pests within a cropping system by allowing Bt-susceptible and Bt-resistant individuals to mate, producing Bt-susceptible heterozygotes (Bates et al. 2005). These tactics, however, would be ineffective against a clonally-reproducing pest. Globally, aphids are perhaps the most impactful group of clonally reproducing crop pests, and have demonstrated a remarkable ability to adapt to control measures and habitat changes (Bass et al. 2014; Mathers et al. 2017). Despite this, no IRM strategies exist for aphid biocontrol.

1.1. Aphids and aphid management in agriculture

Aphids are small, hemimetabolous insects in the superfamily Aphidoidea (Order: Hemiptera). Aphids use piercing-sucking mouthparts to ingest plant sap, and have complex reproduction schemes which often include both sexual and asexual morphs. Aphids are recognized as serious agricultural and horticultural pests largely due to their ability to proliferate rapidly and vector economically important plant viruses. Aphids have also proven to be very resilient in the face of pesticides; the Arthropod Pesticide Resistance Database lists 919 reports of pesticide resistance from 27 aphid species (Mota-Sanchez and Wise 2019). Four hundred and sixty-nine (51%) of these reports represent a single species, the green peach aphid (*Myzus persicae* Sulzer), which has adapted to every major class of pesticide in use today (APRD 2019; Bass et al. 2014).

Life history traits vary greatly between aphid species. For the sake of brevity, I will use *M. persicae* as a model for my review of the aphid life cycle; *Myzus persicae*

shares many life history traits with other agriculturally relevant aphid species in the family Aphididae. The life cycle of *M. persicae* can be considered at the scale of an individual aphid, but also on an annual cycle; these aphids are highly polymorphic, cycling through asexual and sexual morphs in accordance with seasonal changes (Blackman 1971). *Myzus persicae* has one sexual generation per year, taking place in the Fall. Shorter daylight hours in the late summer/early fall prompt the production of winged male and female sexual morphs (the gynoparae; Blackman 1971). Aphids use an XX/X0 sex determination system, where XX individuals are female and X0 individuals are male (Schmidtberg and Vilcinskas 2016). Mated female gynoparae deposit eggs on their primary host, trees in the genus *Prunus* (Davis and Landis 1948). Eggs overwinter in the bark and hatch into wingless nymphs (fundatrices) in the Spring (Davis and Landis 1948). Fundatrices feed on the primary host trees and give birth to viviparae morphs, which may be winged (alate) or wingless (apterous). Alate viviparae will eventually disperse from the primary host to seek out leafy secondary host plants (Davis and Landis 1948). Both fundatrices and vivipara give birth through thyletokous parthenogenesis, where oocytes do not undergo meiosis, and all offspring are female (Schmidtberg and Vilcinskas 2016). Viviparae are born live and give birth to live offspring. They continue to propagate on leafy secondary hosts until shortening daylight cycles once again prompts the production of gynoparae.

Viviparous nymphs have four larval instars, and moult into adults after 6-10 days. Adults have a mean reproductive lifespan of 17 days, and a mean birth rate of four offspring per day, though at peak productivity they can produce up to 10 offspring per day (See Appendix 1). Throughout the Spring and Summer, there are many overlapping generations of genetically identical vivparae. In tropical and sub-tropical regions, populations of *M. persicae* may lose the ability to produce sexual morphs and reproduce exclusively through parthenogenesis (Blackman 1974).

1.2. Biological control of aphids

Various biocontrol agents have been successfully applied against aphids on a wide variety of crops (Powell and Pell 2007). There are three main biocontrol strategies used against aphids: (*i*) classical control, where exotic aphid enemies are imported to new areas, typically to combat invasive aphid pests; (*ii*) augmentative control, where aphid enemies are mass-reared and mass-released into environments where they occur

naturally; and (*iii*) conservation control, where the activity of naturally occurring populations of biocontrol agents is enhanced through habitat or behavioural manipulation. In closed greenhouses, augmentative control is the most common strategy for aphid biocontrol (van Lenteren 2012; Hance et al. 2017).

Aphid predators, such as ladybird beetles (Family Cocinellidae), lacewings (Family Chrysoperidae), predatory midges (Cecidomyiidae: Aphidoletes), and predatory hover flies (Family Syrphidae), are commonly integrated into aphid IPM. With the exception of Aphidoletes midges, predators are typically poor candidates for augmentative control in greenhouses due to their long life cycles relative to aphids, but they remain an important facet of conservation control strategies in other cropping systems (Powell and Pell 2007; van Lenteren 2012).

Several species of Ascomycete and Zygomycete fungi are employed as aphid biocontrol agents and are sold commercially for use in greenhouses (Vu et al. 2007). These fungi are typically applied directly to affected plants as a suspension of conidia, which can be sprayed in the same manner as a chemical pesticide.

Parasitoid wasps in the families Braconidae and Aphelinidae are the most common biological control agents used against aphids (van Lenteren 2012). These konobiont parasitoids are typically applied *en masse* in response to aphid infestations, a tactic termed inundative control. Populations of parasitoids may also be maintained at low levels in greenhouses using a banker plant system, where a secondary host is reared on a non-crop plant within the greenhouse (Powell and Pell 2007; Frank 2010). This tactic allows parasitoids to respond to aphid populations at low levels even before they are detected by growers.

1.3. Aphid defenses and responses to stress

Aphids have developed a wide array of defensive behaviours against parasitoids, including dropping from host plants, wandering, and kicking (Villagra et al. 2002). Aphids may also harbor protective endosymbionts which kill parasitoid eggs and larvae (e.g., Oliver et al. 2009; Vorburger et al. 2010). Notably, aphids do not seem to encapsulate parasitoid eggs (Henter and Via 1995). All these defenses have energetic costs, and have potentially detrimental fitness effects on aphids; for example, aphids dropping from

their host plants risk desiccation and starvation (Roitberg and Myers 1978), and aphids carrying defensive endosymbionts have reduced fecundity (Martinez et al. 2017). These 'non-consumptive' effects can reduce the fitness of an aphid within a single generation (Abram et al. 2019), but can also be passed on to offspring in the form of induced defensive phenotypes.

1.3.1. Inheritance of defensive traits

Defensive traits in aphids can be inherited either through direct genetic inheritance, following the classic Mendelian model of inheritance, or through epigenetic inheritance, mediated by environmentally regulated transgenerational polyphenism.

Currently, there have been only a handful of described resistance traits that are directly inherited across sexual lines. Parasitoid resistance varies across independent genetic lines of the pea aphid (*Acyrthosiphon pisum* Harris), but the exact traits involved in this parasitoid resistance have yet to be explored in-depth. The expression of pigment in *A. pisum* is fixed in independent genetic lines, with some lines being pink and some being green (Tsuchida 2016). Pink *A. pisum* morphs are more susceptible to predation, but less susceptible to parasitoid attack; the inverse is true for green morphs (Losey et al. 1997). Pink *A. pisum* morphs that carry the facultative symbiont *Rickettsiella viridis* lose their pink colouration over the course of their lives (Tsuchida et al. 2010, 2014). Mendelian cross experiments have shown that morph colouration is controlled at a single autosomal locus in this aphid, and that the red allele is dominant to the green allele (Caillaud and Losey 2010).

The production of alate clones is perhaps the most studied defensive trait in aphids. This trait is poly*phenic* rather than poly*morphic*; the expression of the alate phenotype is driven epigenetically, where environmental stressors cause apterous viviparae to produce alate offspring. The molecular mechanisms underlying this polyphenism are discussed in Section 1.4 of this chapter. The ability to produce alate clones in response to stress varies across independent genetic lines (Parker and Brisson 2019). The production of alate clones in various species of aphids has been experimentally induced through simple crowding (reviewed below), predator and parasitoid pressure (Weisser et al. 1999; Kunert and Weisser 2003, Sloggett and Weisser 2002), infection by entomopathpgenic fungi, (Hatano et al. 2012), exposure to

predator kairomones (Dixon and Agarwala 1999; Mondor et al. 2005), aphid alarm pheromone (Kunert et al. 2005), and plant and aphid viruses (Blua and Perring 1992, Ryabov et al. 2009). Within an aphid colony, stress is communicated through the release of the volatile sesquiterpene alarm pheromone E-β-farnesene (EBF).

1.3.2. Aphid alarm pheromones

Aphids release EBF in response to direct stressors, such as predation and parasitism (Vandermoten et al. 2012). EBF is released from the cornicles in small droplets that quickly volatilize. The amount of EBF in these droplets varies by species, ranging between 16 ng and <1 ng (Vosteen et al. 2016). Some aphid species produce other volatile terpenes (Francis et al. 2005), but EBF is by far the most common constituent in stress-induced cornicle secretions (Vosteen et al. 2016). The use of EBF as an alarm pheromone is highly conserved among aphids, although some aphid species do lack EBF (Francis et al. 2005). Whether these aphids have adopted other compounds as alarm pheromones is not yet known.

Exposure to EBF causes aphids to display defensive or dispersal behaviours (Pickett et al. 1992; Andrade and Roitberg 1995; Keiser and Mondor 2013). *Acyrthosiphon pisum* exposed to high doses of EBF produce of alate offspring (Kunert et al. 2005; Podjasek et al. 2005), as well as apterous offspring that disperse from maternal colonies to seek out new feeding sites (Keiser and Mondor 2013). Long-term exposure of *A. pisum* to EBF leads to an attenuation of stress responses (de Vos et al. 2010). Andrade and Roitberg (1995) demonstrated intraclonal variation in *A. pisum* responses to alarm pheromone, and were able to isolate lines of ‘sensitive’ and ‘insensitive’ aphids from a single clonal lineage.

1.3.3. Crowding and pseudo-crowding

Crowding is a major stressor on aphid colonies. Crowded colonies are taxing to their host plants and may accelerate host plant senescence. The buildup of aphid honeydew in large colonies has the potential to attract aphid predators and parasitoids (Hoffmann 2016). When reared under crowded conditions, viviparious aphids produce alate clones; this phenomenon has been demonstrated in many species of the Aphididae, including *M. persicae* (Sutherland and Mittler 1971), *A. pisum*, (Sutherland

1969) *Rophalosiphon padi* L. (Dixon, A.F.G, and Glen 1971), *Megoura crassicauda* (Ishikawa et al. 2012), *Aphis fabae* Scop. (Shaw 1970), *Therioaphis maculate* Buckton (Toba et al. 1967), *Brevicoryne brassica* L., *Megoura viciae* Buckton, and *Aphis craccivora* Koch (Hille Ris Lambers 1966).

The term “pseudo-crowding” was coined by Kunert et al (2005), when they observed that *A. pisum* exposed to EBF produced more alate offspring than unexposed aphids, but only if the exposed aphids were kept in crowded conditions. They suggested that EBF does not directly induce alate offspring production, but instead induces wandering behaviour in exposed aphids. Since increased physical contact between aphids increases production of alate offspring (Lees 1967; Sutherland 1969), Kunnert et al. (2005) hypothesized that, under crowded conditions, wandering aphids would be more likely to produce alate offspring regardless of other stimuli. This hypothesis is further supported by experiments showing that aphids with ablated antennae fail to produce winged offspring in response to predation pressure (Kunert and Weisser 2005). Lees (1967) also demonstrated that crowding-induced alate production in *M. viciae* is not linked to visual or olfactory cues, and could be induced by crowding with other species of aphids (*A. pisum* and *Ap. fabae*), and even non-Hemipteran insects. This begs the question: Are there really multiple discrete triggers for alate production in aphids, or is this phenomenon simply the result of a generalized stress response?

In order to rule out pseudo-crowding as a factor in experiments where transgenerational polyphenism is induced, the experiments must either include aphid density as an explanatory variable or eliminate its effects entirely. This can be accomplished by testing the effects of a given stressor on individual adult aphids (e.g., Mondor et al. 2005), or by replicating the experiment with multiple densities of experimental aphids (e.g. Hatano et al. 2012). It is not sufficient to simply show that aphids under crowded conditions do not produce alate offspring, because it is not the presence of conspecifics that produces a pseudo-crowding effect, but the induction of wandering behaviour in a crowded environment.

Experiments that successfully eliminate or isolate pseudo-crowding effects tell us that there are indeed multiple, discrete triggers for alate production in aphids. For example, when *A. pisum* adults are exposed to entomopathogenic fungi, solitary aphids produce more alate offspring than uninfected controls (Hatano et al 2012). This is good

evidence for a fungus-specific cue that drives transgenerational wing polyphenism. Similarly, cotton aphids, *Aphis gossypii* Glover, exposed individually to kairomones from the ladybird beetles *Hippodamia convergens* Guérin-Méneville produce more alate offspring than unexposed control aphids (Mondor et al 2005). This suggests a predator kairomone-specific cue for transgenerational alate production.

1.4. Molecular mechanisms of transgenerational polyphenism in aphids

While the fitness consequences of certain defensive phenotypes in aphids have been well characterized, the molecular mechanisms underlying the epigenetic control of these phenotypic switches remain largely unexplored.

1.4.1. Comparative genomic techniques in aphids

Comparative transcriptomics is a powerful tool for comparing phenotypes in clonal organisms. In classic comparative genomics, phenotypic differences between individuals are correlated to differences in the sequences of homologous genes (i.e. different alleles of the same gene). When comparing phenotypes arising from a single clonal line, this method falls flat because all the individuals will invariably have identical sequences across their genomes. By instead measuring the mRNA profile, or transcriptome, of aphid clones, we can compare the rates of transcription of specific genes and correlate the changes in rates of transcription to phenotypic differences between individuals.

With the rapid development of next-generation sequencing technologies, there are several options for compiling and comparing aphid transcriptomes. Modern sequencing platforms allow for comparisons of entire transcriptomes (Vellichirammal et al. 2016) at relatively low cost. Alternatively, researchers may use either cDNA microarrays to examine a subset of the aphid genome (Ghanim et al. 2006; Brisson et al. 2007) or target specific genes that have homologs with known functions in other organisms (Brisson et al. 2010). These targeted methods are powerful tools for hypothesis testing and tend to be much quicker than complete transcriptomic analysis. However, the power of these methods as an investigative tool relies on the knowledge

and intuition of the study designer, who may inadvertently overlook genes that have not yet been described, leading to patchy, incomplete models of aphid transcriptomes.

Acyrthosiphon pisum is the most commonly used aphid in comparative transcriptomic studies. This species has long been a model for aphid genomics, and was the first to have it's genome sequenced in 2010 (International Aphid Genomics Consortium 2010) . To date, all comparative transcriptomics studies on this aphid have examined the production of alate offspring in response to crowding. Under crowded conditions, *A. pisum* adults upregulate genes which relate to odorant binding and hormone production/transport, and downregulate genes relating to neurotransmitter transport and chromatin remodelling (Vellichirammal et al. 2016). Alate clones produced in response to a crowded environment upregulate the wing patterning gene apterous1 during the 1st and 2nd instar nymph stages (Brisson et al. 2010). In adult *A. pisum* alates that were produced in response to a crowded environment, genes relating to muscle formation and energy metabolism are upregulated (Brisson et al. 2007).

To date, there have only been a few comparative transcriptomics studies in other aphids. In *M. persicae*, a cDNA microarray identified three genes which are upregulated in alate clones produced in response to crowding (Ghanim et al. 2006). In the vetch aphid, *M. crassicauda*, fluorescence dihybrid display microscopy followed by RT-qPRC allowed Ishikawa et al. (2012) to identify nine genes which are upregulated at least 1.20x in crowded apterous adults.

Ultimately, comparative transcriptomics is an indirect approach for examining polyphenisms; it reveals when transcription is upregulated or downregulated for a gene or sets of genes, but it gives no information on the epigenetic changes to the structure of that genetic material. These epigenetic changes can either occur adjacent to genetic material, as in the case of chromatin remodelling, or directly to the DNA itself, as in the case of DNA methylation.

1.4.2. DNA methylation in aphids

DNA methylation is the only epigenetic modification that involves a direct chemical change to DNA base pairs, where specific enzymes targeting Cytosine-Guanine base pairs catalyze the formation of 5-methylcitosine from cytosine (Lyko

2017). In mammalian models, 5-methylcytosine interacts with transcription factors upstream of coding regions, leading to either upregulated or downregulated expression of the associated genes (Lyko 2017). DNA methylation is conspicuously absent in many insects; this trait appears to have been lost in multiple lineages, including *Drosophila* (Glastad et al. 2019). However, the *A. pisum* genome does contain homologues to the mammalian DNA methyltransferases DNMT1, DNMT2, and DNMT3. If these are functional, they could in theory constitute a complete DNA methylation system (Walsh et al. 2010).

In *M. persicae*, DNA methylation is a contributing factor to insecticide resistance. Upregulation of the modified esterase genes E4 and FE4 is linked to the presence of 5-methylcytosine within the coding regions of these genes (Field and Blackman 2003). Aphids with upregulated E4 and FE4 are able to resist organophosphate, carbamate, and pyrethroid insecticides (Silva et al. 2012). To date, there have been no comprehensive studies of the prevalence of methylated sites across aphid genomes.

1.4.3. The role of aphid hormones

When reared under crowded conditions, viviparous *A. pisum* significantly downregulate the expression of genes involved in the binding of serotonin, dopamine, and octopamine (Vellichirammal et al. 2016). Concentrations of these biogenic amines, as well as the hormone 20-hydroxyecdysone, are reduced in the hemolymph of crowded aphids (Vellichirammal et al. 2016, 2017). Serotonin, dopamine, and octopamine have all been implicated in density-dependent polyphenism in desert locusts, *Schistocerca gregaria* Forsskål (Alessi et al. 2014). Based on these findings, Vellichiramal et al. (2016) proposed a model where sensory inputs associated with crowded conditions cause the aphid brain to stop producing biogenic amines, which leads to a reduction of 20-hydroxyecdysone in the hemolymph.

The role of juvenile hormones (JHs) in wing polyphenism remains unclear. Schwartzberg et al. (2008) demonstrated that pea aphids exposed to larvae of the common green lacewing *Chrysoperla carnea* Stephens produce alate offspring without modifying titres of JH III in their hemolymph. However, Klienjan and Mittler (1975) demonstrated that topical application of the JH analog dendrolasin to 1st instar *Ap. fabae* decreased the likelihood of them developing into alate adults. Interestingly, the jet black

ant, *Lasius fuliginosus* Latr., produces dendrolasin in its mandibular glands, and tending by these ants inhibits alate production in *Ap. fabae* (Klienjan and Mittler 1975). To date, there have been no transcriptional analyses of JH-activated genes or gene pathways in any aphid species.

1.4.4. The role of aphid viruses

Genetic lines of *A. pisum* vary in their ability to produce alate offspring in response to crowding stress (Parker and Brisson 2019). In *A. pisum*, higher "inducibility" is correlated with an upregulation of two genes, Apns-1 and Apns-2. These genes were acquired by *A. pisum* through a lateral transfer, likely from the rosy apple aphid densovirus (DpIDSV) (Parker and Brisson 2019). DpIDSV was first isolated from the rosy apple aphid, *Diaphis plantaginea*; these aphids produce alate offspring in response to crowding, but only if they are infected with DpIDSV (Ryabov et al. 2009). A similar densovirus has been isolated from *M. persicae,* but its potential contribution to wing polyphenism remains unclear (van Munster et al. 2003).

The case of Apsn1 and Apsn2 is the second report of *A. pisum* relying on laterally transferred genes to generate a defensive phenotype. Pink *A. pisum* clones obtain their colouration from a variety of carotenoid pigments that are produced via the cyclization and oxidation of terpenoid precursors; no less than seven of the enzymes involved in these pathways have been inherited via lateral transfer from a fungal genome (Moran and Jarvik 2010). Currently, the traits arising from these laterally-transferred genes are the only defensive traits which have been linked to specific loci.

1.5. 1.5 Biological study system

1.5.1. The green peach aphid, *Myzus persicae* Sulzer

Myzus persicae is considered one of the most important aphid pests in agriculture (Blackman and Eastop 2017). This aphid uses over 120 plant species in 40 families as secondary hosts (CABI 2013). Of these 120 reported host species, 90% are cultivated crops or ornamentals, suggesting that the true number of host plant species is massively underestimated in the literature. *Myzus persicae* is capable of host-plant switching and adapting to new metabolic requirements within a single asexual

generation (Mathers et al. 2017). However, host-adaptation does occur, as is the case in *Myzus persicae ssp*. *nicotinae,* which reproduces exclusively on tobacco (Margaritopoulos et al. 2003). Perhaps the most immediate threat *M. persicae* poses to growers is as a viral vector; this aphid has been reported to vector over 100 species of plant virus (Blackman and Eastop 2017).

I chose *M. persicae* as my model aphid due to its immediate relevance as an agricultural pest and its remarkable ability to adapt to environmental stressors using transgenerational polyphenisms (Kaiser 2017; Mathers et al. 2017). I believe that a more holistic understanding of how these polyphenisms interact with biocontrol measures is necessary for the development of comprehensive integrated programmes for *M. persicae* management.

1.5.2. The aphid parasitoid, *Aphidius colemani*

For my study, I chose *Aphidius colemani* (Family: Braconidae) as the model biological control agent because of its commercial availability and popularity as a non-specific biological control agent for aphids (van Lenteren 2012).

The life cycle of *A. colemani* resembles that of most braconid wasps. Adult females reproduce by injecting a single egg into an aphid host. This egg hatches into a larva which consumes its host from the inside out within 6-8 days. Once larval development is complete, the host aphid dies and its body hardens into a shiny, gold-coloured “mummy”, inside which the parasitoid larva spins a cocoon and undergoes metamorphosis. Within 6-8 days, an adult parasitoid chews its way out of the mummy and continues the cycle.

1.6. Does transgenerational polyphenism help protect aphids from parasitoid wasps?

Aphid defensive polyphenisms have a serious potential to disrupt biocontrol efforts. So far, the production of alate offspring by apterous viviparae is the only biocontrol-induced defensive polyphenism described in aphids relevant to Canadian agriculture. Recent genomic and experimental evidence suggests there may be more subtle polyphenisms available in the aphids’ toolkits, but there has been little research

explicitly studying this. Based on the evidence provided by Kaiser (2017) and Mathers et al. (2017), I hypothesize that aphids may adapt to biological controls over multiple, consecutive clonal generations using polyphenisms that manifest both as visible phenotypes and changes in aphid reproductive schedules. To test this hypothesis, I designed an experiment that combined traditional experimental evolution methodology (eg Sloggett and Weisser 2002; Sentis et al. 2018).

In order to elicit defensive polyphenisms in experimental aphids, I isolated a single genetic line of aphids and exposed four consecutive generations from this genetic line to a standardized parasitoid pressure, controlling for any effects of pseudo-crowding. If we observe that *M. persicae* adapts to *A. colemani* over consecutive exposures, this finding could have implications for how *A. colemani* is applied to these aphids as a biocontrol agent. We may recommend that growers rotate biocontrol agents, or apply multiple agents simultaneously to prevent the establishment of resistant aphid lines.

1.7. References

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Chapter 2. Does parasitoid pressure elicit defensive polyphenism in the green peach aphid?*

*A near identical version of this chapter has been submitted to *Ecological Entomology* with the following authors: Yonathan Uriel, Paul Abram, and Gerhard Gries. Submitted May 25th, 2020, currently under review.

2.1. Introduction

Pest management strategies should account for the likelihood that pests will develop resistance to control measures. Development of resistance is an especially pressing issue in the management of arthropods; their short life cycles accelerate the selection of resistance genes, and the continued distribution of pests through flight or anthropogenic means facilitates the flow of resistance genes across wide areas (e.g., Dunley and Croft 1992; Pasteur et al. 1995). High-dose/refuge cropping in Bt crops is a successful example of insect resistance management in agriculture; this strategy takes into account both the likelihood of resistance genes developing and how they may spread within a semi-resistant population (Bates et al. 2005; Camargo et al. 2018). Resistance management increases the resiliency of pest management programs, which may lead to more widespread adoption of sustainable farming practices.

Many arthropod pest management tactics lack a framework for resistance management, including the biological control (biocontrol) of insects using other arthropods. For example, insect biocontrol programs have not historically practiced post-release monitoring, which is needed for tracking the development of resistance (Barratt *et al.*, 2006; Van Driesche & Hoddle, 2016), nor has the field of biological control developed a clear framework for understanding when and how arthropod pests could become resistant to biocontrol agents. The conventional view seems to be that co-evolutionary relationships between biocontrol agents and insect pests hinder the development of resistance. This assumption may be valid for open cropping systems that rely on conservation biocontrol (i.e. the implementation of practices that maintain and enhance the reproduction, survival, and efficacy of natural enemies), but may not apply to augmentative and inundative biocontrol tactics that rely on transient populations of control agents, where co-evolutionary relationships between control agents and pests

are unlikely to evolve (Bale *et al.*, 2008). Cases of insect pests becoming resistant to biocontrol agents are rare, but may become more common as a consequence of intensified agriculture (Tomasetto *et al.*, 2017). A robust theoretical framework for preventing arthropod resistance to biocontrols would necessarily be complex and multi-dimensional, including a detailed understanding of the biology and ecology of the specific crop, the pest(s), biocontrol agent(s), and all possible interactions between them within a given system. In addition, there must be a full understanding of the methods by which pests may gain resistance to biocontrol agents; for example, genetic variation is usually thought to be the raw material for natural selection to produce resistant phenotypes, but clonal organisms may also develop resistance through epigenetic plasticity.

Aphids (Hemiptera: Aphidoidea) are cosmopolitan, phloem-feeding, plant-parasitic insects that cause severe damage in field and greenhouse crops. Aphids vector hundreds of economically important species of plant virus (Ng & Perry, 2004), and large colonies debilitate young plants and new growth through excessive phloem-feeding and the encouragement of sooty mold growth (Smith, 1997; Dedryver *et al.*, 2010). Aphids have two key reproductive strategies that make them a challenging pest to manage: asexual reproduction and transgenerational polyphenism. Asexual reproduction, accomplished through thyletokous parthenogenesis (Schmidtberg & Vilcinskas, 2016), typically occurs under greenhouse conditions. This reproductive strategy is extremely efficient in that aphids begin developing embryos in their ovaries before they themselves are born – this allows females reaching adulthood to immediately begin giving birth, producing multiple offspring per day for the duration of their reproductive lifespans (Schmidtberg & Vilcinskas, 2016). As a result, aphid colonies grow at near exponential rates, and a colony can be established by a single individual. Polyphenism is the ability to produce multiple phenotypes from a single genotype through epigenetic modification of gene expression. In aphids, this manifests as a transgenerational defensive strategy; naïve aphids can induce the development of novel phenotypes in their unborn clonal daughters using hormone-triggered epigenetic switches (Vellichirammal *et al.*, 2017). This may occur in response to aphid alarm pheromone (Kunert *et al.*, 2005), host-plant toxins (Mathers *et al.*, 2017) or the presence of predators (Dixon & Agarwala, 1999; Kunert & Weisser, 2003; Mondor *et al.*, 2005; Sentis *et al.*, 2018), parasitoids (Sloggett & Weisser, 2002), pesticides (Field and Blackman 2003), and pathogens (Ryabov *et al.*,

2009; Hatano *et al.*, 2012). Paired with rapid reproduction, these transgenerational polyphenisms enable aphids to rapidly adapt to environmental stressors, possibly including natural enemies applied as biocontrol.

Aphids are prime targets of biocontrol programs in protected agriculture (Powell & Pell, 2007) and parasitoid wasps are the most common biocontrol agents used against them (van Lenteren, 2012). Thus far, there are no reports of aphids developing widespread resistance to parasitoid wasps within a cropping system, but aphid management protocols should still account for this possibility. There are at least three potential mechanisms by which aphids could increase their resistance to parasitoids: (*i*) selection on pre-existing genetic variation within a population (Losey *et al.*, 1997; Martinez *et al.*, 2014); (*ii*) recruitment of protective gut symbionts (Oliver *et al.*, 2014); and (*iii*) epigenetically controlled transgenerational polyphenisms within clonal lines.

Epigenetically controlled parasitoid-induced polyphenisms have been described in several species of aphids. In response to parasitoid pressure, wingless (apterous) adult pea aphids, *Acyrthosiphon pisum* Harris, produce winged (alate) offspring (Sloggett & Weisser, 2002) which have lower reproductive output but can fly to new host plants (Muller et al 2001). As an alternate response to parasitoid pressure, the soybean aphid, *Aphis glycines* Matsumura, produces offspring with increased reproductive output (Kaiser 2017). Both strategies help increase the fitness of a clonal line within a single asexual generation. Other aphid traits such as body size and pigmentation affect parasitism success (Losey *et al.*, 1997; Gwynn *et al.*, 2005) but have not yet been linked to polyphenisms induced in response to parasitoid stress. Understanding the breadth of strategies aphids can use to combat parasitoids is essential to the design of resilient IPM programs against these insects.

Working with the green peach aphid, *Myzus persicae* Sulzer, and the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) as our model aphid-parasitoid system, our objective was to induce and measure defensive polyphenisms using a rigorous, replicated experimental evolution approach. Among aphids, *M. persicae* has an exceptional ability to adapt to control measures, accounting for 461 of the 919 incidences of pesticide resistance in aphids reported in the Arthropod Pesticide Resistance Database, with reports spanning every major class of modern pesticide (Mota-Sanchez & Wise, 2019). In keeping with this phenotypic flexibility, *M. persicae* has

the largest documented host-plant range of all aphids, being able to reproduce on over 100 plant species in >40 plant families (CABI, 2013; Blackman & Eastop, 2017), and to readily switch hosts within a single asexual generation by epigenetically modifying the metabolism of developing embryos to better suit novel host plant defenses (Mathers *et al.*, 2017). *Myzus persicae* is a serious pest in multiple crops due to its ability to vector over 100 species of economically important plant viruses (Blackman & Eastop, 2017). *Aphidius colemani* is a commercially available aphid generalist and is commonly used as an aphid biocontrol agent in greenhouses, including against *M. persicae*.

We tested the hypothesis that adult *M. persicae* exposed to *A. colemani* will produce offspring with adaptive phenotypes, and with an increased resistance to *A. colemani.* This hypothesis entailed five specific predictions:

Prediction 1: Offspring of stressed M. persicae suffer reduced mortality following parasitoid exposure

Aphids do not encapsulate parasitoid eggs (Henter & Via, 1995) but some aphid genomes carry homologs for *Drosophila* immune-response genes induced by parasitoid attack (Gerardo *et al.*, 2010). Thus, we predicted that *M. persicae* under parasitoid stress will produce offspring with better defenses against immature parasitoids, characterized by either a longer latency period between parasitoid oviposition and mummification (Niogret *et al.*, 2009), or increased survival following parasitoid exposure.

Prediction 2: Offspring of stressed M. persicae have an accelerated reproductive schedule

Kaiser (2017) demonstrated that, when aphids are under parasitoid stress, their offspring produce a higher mean number of offspring over their reproductive lifespan. At the time of this study, other aspects of the aphids' reproductive schedule in response to parasitism across generations remained unexplored. We predicted that *M. persicae* under parasitoid stress will produce offspring with an accelerated reproductive lifespan, reflecting a shift in resource allocation from growth and development to reproductive output (Niogret *et al.*, 2009; Frost *et al.*, 2010).

Prediction 3: Offspring of stressed M. persicae have an increased body size

In direct response to parasitoid attack, aphids kick at their attackers, wander away from feeding sites, and drop off their host plants (Villagra *et al.*, 2002). Aphid body size correlates with the lengths of their hind tibiae (Gwynn *et al.*, 2005) which may correlate with greater success in these defensive behaviours. Accordingly, we predicted that *M. persicae* under parasitoid stress will produce offspring with larger body size (longer tibiae).

Prediction 4: Stressed M. persicae produce more pink offspring than naïve aphids

Myzus persicae exhibits colour diphenism with two distinct clones: green and pink (Tsuchida 2016; Fig. 1). In our laboratory colony, green clones produce pink offspring under crowding stress (Fig. 1), and pink clones are more likely to develop into alate adults than green clones. The pea aphid, *Ac. pisum,* exhibits colour polymorphism with similar pink and green morphs. In this aphid, green morphs are more susceptible to parasitism by the wasp *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) than the pink morphs (Losey *et al.*, 1997). Thus, we predicted that green morphs of *M. persicae* would produce pink morphs under parasitoid pressure.

Prediction 5: Stressed M. persicae produce more alate offspring than naïve aphids

Myzus persicae produce alate offspring under crowding stress (Sutherland, 1969). This stress response is highly conserved between aphid taxa, and appears to be triggered by various stressors. As apterous soybean aphids, *Ap. glycines*, produce alate offspring in response to parasitoid attack (Kaiser 2017), we predicted that the same stress response would be present in *M. persicae*.

Figure 1: Polyphenism in Myzus persicae. All individuals in this photo are descended from the same genetic lineage, their distinct phenotypes (from left to right: alate, apterous, and pink) were produced as the result of crowding stress in our research colony. Photo by Warren Wong, used with permission.

2.2. Methods

2.2.1. Aphids

All of the *M. persicae* used in our study were descendants from a single first instar aphid taken from a long-standing colony maintained in the Insectary at AAFC Agassiz. Selecting a single aphid for our experiment as the progenitor of all aphids ensured they were identical genetic clones and controlled for possible effects of clonal lineage. Colony aphids were reared on potted bell pepper plants inside BugDorm tents (BioQuip Products, 2321 Gladwick St. Rancho Domingues, CA, USA) at 19 °C (± 2 °C) and 60% (± 5%) humidity on a 16:8 h light-dark cycle. Maintenance of aphids at a constant temperature, humidity, and light cycle ensured that they produced only parthenogenetic, viviparous offspring. To keep aphid colonies at low density, we replaced old plants with new plants once per week, transferring ~20 aphids to each new plant. Abiotic conditions for the experiments described below were identical to those used for rearing.

2.2.2. Parasitoids

We purchased *Aphidius colemani* Viereck from Westgrow Biological Solutions (Fort Langley, BC, Canada) and reared all specimens used in our experiments inside BugDorm tents provided with *M. persicae*-infested pepper plants and water *ad libitum*. The tents were kept in the AAFC Insectary, using the same abiotic conditions as detailed for *M. persicae* (see above). To select experimental parasitoids, we isolated parasitized aphid mummies from our colony into a separate BugDorm cage fitted with a pepper plant and a water wick. Within 24 h of emergence, parasitoids were separated by sex and isolated into 120 mL plastic screw-top containers (Thomas Scientific, Swedesboro NJ, USA) with a water wick. As a result, all parasitoids used in our experiment were the same age. After 48 h in the screw-top containers, we collected female parasitoids for use in experimental assays.

2.2.3. Multi-generation exposure of aphids to parasitoids

We tested the five predictions listed above in a single experiment in the AAFC Insectary. To initiate the experiment, one adult female aphid was allowed to reproduce on an intact pepper plant inside a Bugdorm tent. First-instar offspring of this aphid (F1) were placed on a single pepper leaf (n = 60) with its petiole inserted through a hole in the bottom of a 30 ml plastic cup (Unisource Canada, Delta BC, Canada) nested - at water level - inside a 120 ml Styrofoam cup (Loblaw Co. Ltd., Brampton ON, Canada) containing 30 ml of water. This design ensured that the pepper leaf was well watered but that water did not enter the remainder of the Styrofoam cup. We covered these Styrofoam cups with a single Kimwipe secured by a rubber band. Pepper leaves inside these cups remained viable for up to six weeks.

Each experimental replicate started with a group of 15 treatment and 15 control first-instar F1 aphids. Once they had become adults (monitored every 48 h), we selected 10 treatment and 10 control aphids and placed them on pepper plants in separate 30 cm^3 cages (BioQuip BugDorm 1). Following 48 h of acclimation, we removed all offspring that they had already produced and released three mated *A. colemani* females into the treatment cage, allowing host seeking for 10 h; no parasitoids were released into the control cage. Subsequently, we removed aphids from the cages, placed them singly on a pepper leaf in a Styrofoam cup (see above), and every 48 h recorded the number of

offspring produced, and any mummification that occurred. We further noted the time elapsed between mummification and parasitoid emergence. Forty-eight hours post parasitoid exposure, we randomly selected five aphids each from the treatment and the control group, and collected three first-instar offspring (F2) from each aphid, for a total of 15 first instars per group. These F2 aphids constituted the second generation of experimental aphids. Once they became adults, they were subjected to the same experimental treatment as their parents (as detailed above). Because we did not track the parentage of each offspring, generations F2-F4 could be considered “mixed-stress” groups, where individual aphids may be the offspring of adults who were directly attacked by a parasitoid, or were stressed indirectly – experiencing non-consumptive effects (Abram et al. 2019).

To quantify transgenerational effects of parasitoid exposure on aphid reproductive schedules in each generation, we kept the remaining F1 treatment and control aphids that had not been exposed to parasitoids in Styrofoam cup chambers (see above) and recorded at number of offspring produced every 48 hours for the remainder of their reproductive lifespans. All offspring were removed every 48 hours to prevent crowding. We considered aphids to have reached the end of their reproductive lifespan when they ceased producing offspring for 96 h. At this time, we measured the length of one of the aphids’ hind tibia as a proxy for body size.

Repeating this process with F2 aphids, their offspring (F3), and with the offspring of the F3 aphids (F4), we generated data with four consecutive generations on parasitoid resistance, reproductive schedule, and body size. We replicated this assay a total of four times, with each replicate representing a separate “population” of aphids. Replicates were split into time blocks, with two replicates per block. This constitutes a standard experimental evolution design, with each population representing a single experimental unit, while measurements are pseudo-replicated many times within each replicate (Lirakis & Magalhães, 2019).

2.2.4. Statistical analysis

Our experiment included four complete replicates, each with a treatment and a control group. Each group represented a single genetic line of aphids, which we followed over the course of four consecutive generations. Thus, our experiment consisted of four

sets of repeated measures (one per generation for four generations) across a total of eight separate genetic lines of aphids. To account for any variation between genetic lineages, all our statistical models were run as mixed-effect models, with replicate as a random effect. However, in all cases, this resulted in models with singular fits, indicating that the models were overfitted as a result of experimental replicate having no effect on the response variables. Thus, we chose to discard replicate as a random effect and ran simple fixed-effect models, as recommended by Barr et al. (2013). All statistical analyses were performed in R for Windows (R Core Team 2018).

Prediction 1: Offspring of stressed M. persicae have increased parasitoid resistance

To measure changes in parasitoid resistance over four experimental generations, we compared the proportion of aphids in each treatment group that mummified after parasitoid exposure using a binomial generalized linear model followed by an ANCOVA. We took the proportion of parasitized aphids as the response variable, generation as a continuous effect, and time block as a blocking factor.

To measure changes in parasitoid development over four experimental generations, we compared the time elapsed between parasitoid exposure and aphid mummification, and the time elapsed between aphid mummification and parasitoid emergence using linear models followed by one-way ANOVAs. Each model included generation as a continuous fixed effect factor, and time block as a blocking factor.

Prediction 2: Offspring of stressed M. persicae have an accelerated reproductive schedule

To measure potential changes in aphid fecundity in response to parasitoid exposure across four experimental generations, we compared the total number of offspring produced by aphids under control and treatment conditions post parasitoid exposure using a linear model followed by an ANCOVA. We took generation and treatment as fixed effect variables, and time block as a blocking factor.

A typical reproductive curve for our lab aphids is shown in Figure 2. We used this curve as a model when designing our life history analysis. Based on the bimodal distribution of the curve, and the downwards slope of both ends, we determined that aphid reproductive output over time would be best modeled as a fourth-order polynomial.

We measured the timing of peak reproduction for each treatment group by fitting reproductive curves to fourth-order polynomials, and then extracting the maximum value of the polynomial. These maximum values were compared between treatment and control aphids across all four generations using a linear model followed by an ANCOVA, with generation and treatment as fixed effect variables and time block as a blocking factor.

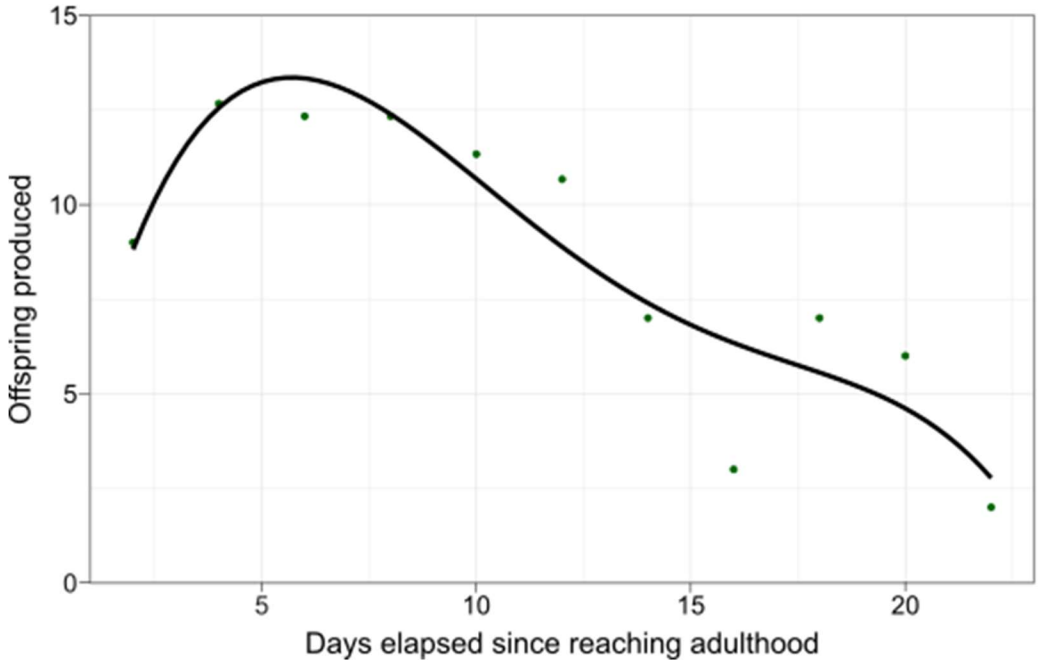

Figure 2: Mean reproductive output (recorded every 48 h over the entire reproductive lifespan) of all the green peach aphids used in our study. The black line depicts a LOESS curve of the data generated for ease of visualization. Note the skewed character of the curve, and the slight bimodal distribution.

Prediction 3: Offspring of stressed M. persicae have an increased body size

To measure changes in aphid body size in response to experimental treatment, we compared hind tibia length (a proxy measure) of adult aphids under treatment and control conditions post parasitoid-exposure using a linear model followed by an ANCOVA. We took generation and treatment as fixed effect variables, and time block as a blocking factor.

2.3. Results

Prediction 1: Offspring of stressed M. persicae have increased parasitoid resistance

Analysis of aphid mortality following parasitoid exposure revealed no change in proportion of mummified aphids per treatment group (Likelihood-ratio $\chi^2 = 0.79$, df = 1, p = 0.11; Fig. 3), time elapsed between parasitoid exposure and mummification (ANOVA, $F_1 = 0.71$, p = 0.42, Fig. 4A), or time elapsed between mummification and parasitoid emergence (ANOVA, $F_1 = 0.03$, p = 0.87, Fig. 4B) across four experimental generations.

Prediction 2: Offspring of stressed M. persicae have an accelerated reproductive schedule

Analysis of reproductive schedules of aphids across four experimental generations showed no effect of experimental treatment on the total number of offspring produced (ANCOVA, $F_{1,24} = 0.28$, p = 0.61; Fig. 5A) and no effect on the timing of peak reproduction across four experimental generations (ANCOVA, $F_{1,24} = 0.05$, p = 0.82; Fig. 5B).

Prediction 3: Offspring of stressed M. persicae have an increased body size

Analysis of adult aphid tibial length across four experimental generations showed no effect of experimental treatment on adult aphid body size (ANCOVA, $F_{1,12} = 0.76$, p = 0.40; Fig. 5C). However, aphid body size did increase throughout our experiment in both treatment and control groups (ANCOVA, $F_{3,12} = 9.39$, p = 0.002; Fig. 5C).

Prediction 4: Stressed M. persicae produce more pink offspring than naïve aphids

Throughout the entire experiment, no pink offspring were produced.

Prediction 5: Stressed M. persicae produce more alate offspring than naïve aphids

Throughout the entire experiment, no alate offspring were produced.

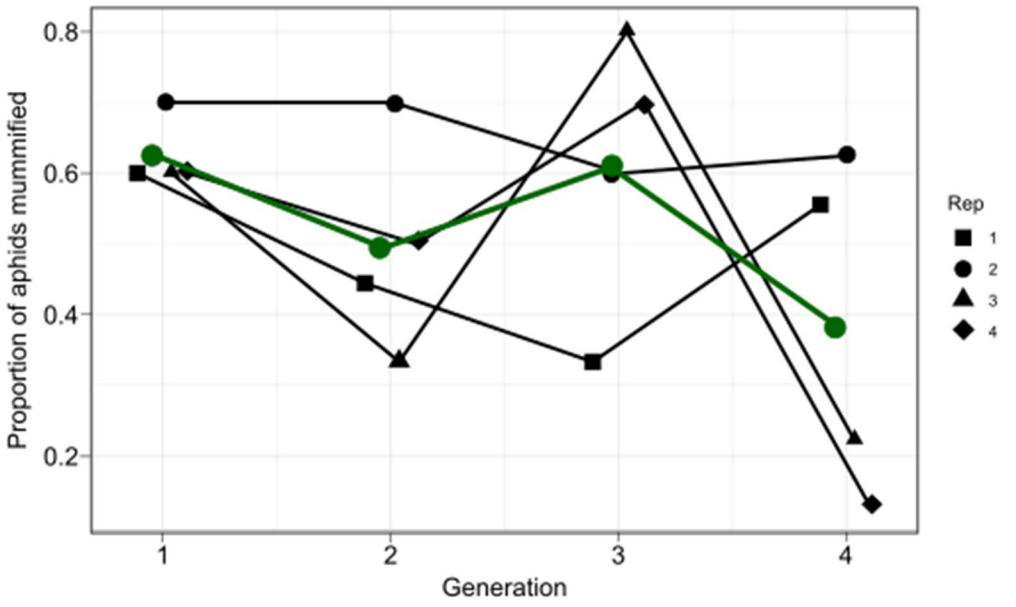

Figure 3: The proportion of green peach aphids, *Myzus persicae*, parasitized (mummified) by the parasitoid *Aphidius colemani* across generational lines in each of four experimental replicates (Reps) (black lines), and the mean number of parasitized aphids in each generation (green line). A binomial GLM followed by an ANCOVA showed no significant change over the four experimental generations (Likelihood-ratio χ^2 = 0.79, df = 1, p = 0.11).

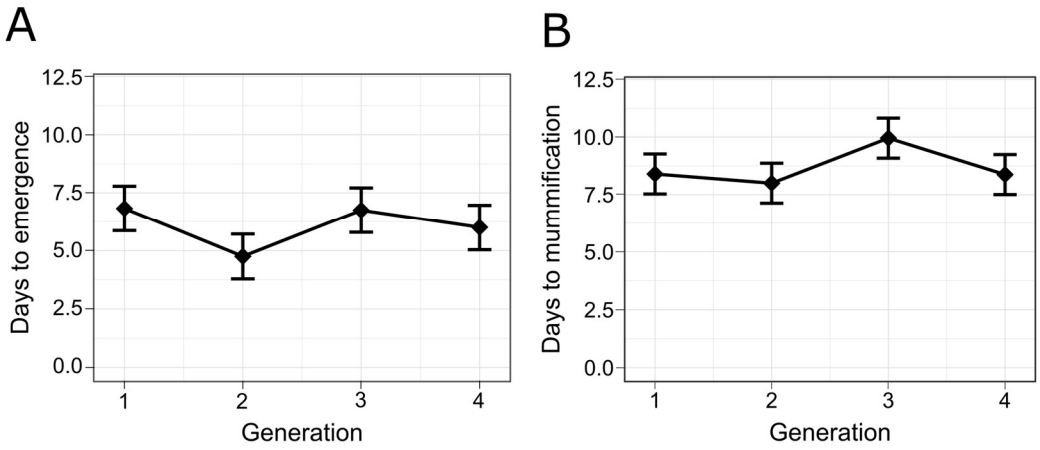

Figure 4: The mean number (± standard deviation) of days elapsed (A) between exposure of green peach aphid*s*, *Myzus persicae*, to the parasitoid *Aphidius colemani* and aphid mummification (ANOVA, F_1 = 0.71, p = 0.42), and (B) between mummification and the emergence of *A. colemani* from mummies (ANOVA, F_1 = 0.03, p = 0.87), all across four experimental generations.

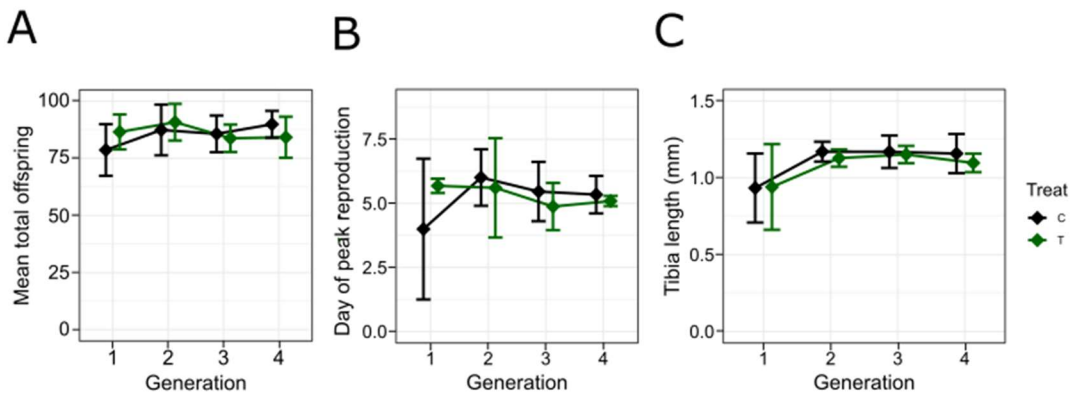

Figure 5: (A) The mean total number of offspring (± standard deviation) produced by treatment (T) and control (C) green peach aphid*s, Myzus persicae,* over their reproductive lifespans (ANCOVA, $F_{1,24} = 0.28$, $p = 0.61$); (B) The mean number of days (± standard deviation) between the final moult and peak reproduction in treatment and control aphids (ANCOVA, $F_{1,24} = 0.05$, $p = 0.82$); and (C) the mean tibia length (mm) (± standard deviation) of treatment and control aphids (ANCOVA, $F_{1,12} = 0.76$, $p = 0.40$).

2.4. Discussion

Taken together, our experimental data provide no evidence that *M. persicae* is capable of producing offspring with defensive phenotypes in response to parasitoid exposure. This finding is surprising because *M. persicae* does respond to other stressors by producing morphs with adaptive phenotypes, such as (*i*) alate offspring in response to crowding (Sutherland, 1969) and predation (Sentis *et al.*, 2018), and (*ii*) offspring with modified metabolic profiles in response to host-plant switching (Mathers *et al.*, 2017). In our lab colony of *M. persicae*, pink and alate clones are often produced, probably in response to crowding stress (Fig. 1) and in other aphid species, alate offspring and offspring with modified reproductive rates are produced in response to parasitoid pressure (Sloggett and Weisser 2002; Kaiser 2017). These defensive polyphenisms are widely reported, and are an important part of aphid stress biology, but as our data show, they are not inevitable, even under conditions of high parasitism pressure over multiple generations.

There is no obvious explanation why – contrary to our prediction – experimental aphids failed to produce defensive phenotypes in response to parasitoid exposure, even though aphids interacted with parasitoids, and parasitoids caused a 53% mean mortality across four experimental aphid generations. As genetic lines of aphids vary in their ability to produce defensive polyphenisms (Braendle *et al.*, 2005), it is conceivable that

our lab strain of *M. persicae* is particularly insensitive to parasitoid stress. Ambient lab conditions may also have contributed to our results; Williams et al. (2000) found that, under laboratory conditions, *M. persicae* withstood much higher crowding stress before producing alate offspring than previously observed in natural settings. Is also possible that our lab colony of *M. persicae* has adapted to laboratory conditions at the expense of typical phenotypic plasticity. Intuitively, one may not expect this to be possible in an asexually reproducing colony, but aphid clonal lines do not have fixed genotypes; *M. persicae* chromosomes are more susceptible to fragmentation and rearrangement than those of other insects (Monti *et al.*, 2012; Mandrioli *et al.*, 2019), and even moderate mutation rates are compounded by the exponential growth of aphid colonies (Loxdale *et al.*, 2020). We expect that our colony could have developed several variant genotypes somewhere among the estimated 50 million embryos[1] produced since colony establishment. Maintaining an aphid colony also involves regular bottleneck events, where only a small subset of aphids are transferred from infested old host plants to new host plants. This type of transfer could potentially select for any number of behavioural/physiological traits even in the absence of sexual reproduction (Andrade & Roitberg, 1995).

Wing polyphenism and reproductive polyphenism are currently the only two transgenerational phenomena characterized as adaptive responses to biological control agents by aphids (Sloggett & Weisser, 2002; Kaiser, 2017). While these adaptions are effective tools for parasitoid evasion, aphids have many defensive behaviours and traits that are not linked to flight (alate offspring) or rapid reproduction. In response to chemical defenses of a new host plant, adult *M. persicae* alter the metabolism of their offspring *in utero* (Mathers *et al.*, 2017), and in response to predation cues, pea aphids exhibit complex dropping and piggybacking behaviour (Gish & Inbar, 2018). If the epigenetic processes that engender the production of alate offspring can also prompt changes in metabolism and behaviour, it is feasible that *M. persicae* may be able to alter other aspects of its biology, such as immune responses or even changes in gut microbiome composition. There have already been several studies into the molecular nature of aphid adaptive polyphenisms, using comparative transcriptomic techniques to determine the genes that are under epigenetic control. These analyses can target

[1] 15 years * 365 days/year * 10 embryogenesis events/adult aphid/day * 1000 (approx.) adult aphids in a mature colony.

specific genes and gene families, using tools such as DNA microarrays or RT-qPCR (Brisson *et al.*, 2010; Ishikawa *et al.*, 2012), or could be genome-wide association studies, using next-generation sequencing technology to produce complete transcriptomes (Vellichirammal *et al.*, 2016; Mathers *et al.*, 2017). Future work in this area would benefit greatly from incorporating genomic analysis, as it provides additional context to known aphid behaviours and stress-responses and may reveal novel aspects of aphid stress biology.

One unexpected result from our experiment was the increase in aphid body size that occurred over the course of our experimental generations in both the treatment and control groups. This result may be attributable to contrasting density conditions in our lab colony and the experimental setup. Over the experimental period, aphids were kept at a very low density, with each adult occupying a separate Styrofoam cup and with all offspring removed every 48 h. In our lab colony, a single host plant may host hundreds of aphids at a time. Since aphid body size increased gradually throughout our experiment, we conclude that green peach aphids in low-density colonies produce offspring with a larger body size than aphids in high-density (crowded) colonies. This may be due to limited resources – denser colonies reduce host plant quality (Smith & Schowalter, 2001) and may increase instances of predation or parasitism due to honeydew deposition (Hoffmann, 2016) – or it may be a form of density-dependent adaptive polyphenism, where aphids produce smaller offspring in a crowded environment in order to reduce strain on the host plant and increase the effective lifespan of the colony.

Crowding is a well-documented stressor on aphids; in many species, apterous viviparae – when crowded – produce alate offspring (Hille Ris Lambers, 1966; Toba *et al.*, 1967; Sutherland, 1969; Shaw, 1970; Sutherland & Mittler, 1971; Ishikawa *et al.*, 2012), prompting the same stress response triggered otherwise by predation (Weisser *et al.*, 1999; Kunert & Weisser, 2003) or parasitism (Sloggett & Weisser, 2002). However, many studies of wing polyphenism as an aphid stress response do not account for aphid density. Therefore, the interpretation of data could possibly have been confounded by a pseudo-crowding effect, where disturbed aphids move around within a colony, resulting in more aphid-to-aphid contact, which mimics a crowded environment (Kunert *et al.*, 2005). Our study design specifically eliminated any possible pseudo-crowding effects by keeping all experimental aphids at very low density. Although not realized in previous

studies, our data indicate that pseudo-crowding stress may be necessary for *M. persicae* to produce defensive polyphenisms in response to parasitoid attack.

In summary, our results demonstrate that parasitoid pressure alone is not sufficient to generate defensive polyphenisms in *M. persicae*. However, we do not exclude the possibility that this lack of an adaptive response may have been due to strain-specific insensitivity to parasitoid stress, or due to confounding effects arising from the laboratory setting for our experiment. Future studies may be able to reveal defensive polyphenisms in *M. persicae* using larger-scale experiments (e.g., Sentis et al. 2018) or comparative transcriptomics approaches (Brisson *et al.*, 2007; Mathers *et al.*, 2017). By including aphid density as an experimental variable, future studies may also conclusively demonstrate a link between parasitoid pressure, crowding stress, and defensive polyphenism in aphids.

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Chapter 3. Producing a high-quality draft genome assembly* of the green peach aphid "Gillespie clone" using PacBio long-read sequencing

*To be made publicly available on GenBank by Dec. 2020.

This chapter was produced in close collaboration with David A. Kudrna and Seunghee Lee from the Arizona Genomics Institute, School of Plant Sciences, Arizona State University. David and Seunghee performed the DNA extraction and PacBio sequencing.

3.1. Introduction

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is a globally-distributed crop pest, capable of reproducing on over 100 economically important crop plant species (CABI 2013). In fact, this aphid may have the widest host-plant range of any animal, with over 400 recorded host plant species distributed between 40 plant families (Blackman and Eastop 2017). This species has the most records of novel instances of pesticide resistance of any aphid (Mota-Sanchez and Wise 2019), enhancing its severity as a crop pest.

Myzus persicae relies on epigenetically controlled transgenerational polyphenism to adapt to environmental stress. Adults reproduce primarily through apomicitic thyletokous parthenogenesis, and react to adverse environmental conditions by sending hormonal signals to developing clonal embryos (Vellichirammal et al. 2017). These signals lead to epigenetic modifications of gene expression in the embryos, enabling *M. persicae* to produce multiple phenotypes within a single asexual line. These phenotypes can be specialized for dispersal (Sutherland and Mittler 1971), reproduction (Kaiser 2017), pesticide resistance (Field and Blackman 2003), or feeding on toxic host plants (Mathers et al. 2017). This phenotypic variability, paired with rapid reproduction, enables *M. persicae* to both quickly multiply within greenhouses and field crops and to adapt to control efforts. In recent years, genetic and genomic analyses have significantly improved our understanding of these polyphenisms (e.g., Mathers et al. 2017).

In addition to this epigenetic flexibility, the genome structure of *M. persicae* can vary significantly within a single clonal lineage. Chromosomes of *M. persicae* are fragile

and prone to fragmentation and rearrangement (Sunnucks et al. 1996; Monti et al. 2012; Mandrioli et al. 2019). These kinds of genomic structural variants (SVs) have been linked to pesticide resistance in *M. persicae*, as exemplified by the carboxylesterase gene E4*,* which is amplified in resistant aphids due to a chromosomal translocation (Field and Blackman 2003). Aphid genetic lines may also be prone to intra-clonal single nucleotide polymorphisms (SNPs); even with conservative estimates of standing mutation rates, the exponential growth of asexual colonies may allow many point mutations to accumulate within a single asexual lineage over time (Loxdale et al. 2020).

Genetic and genomic analyses have greatly enhanced our understanding of the breadth and complexity of aphid resistance to control measures. These analyses rely on high-quality reference genomes. Currently, a number of aphid genomes, assembled from both long-read and short-read sequencing data, have been published for multiple species (Table 1).

To date, all available *M. persicae* genome assemblies have been completed with Illumina short-reads. In addition to being cheaper to produce, short-read data sets typically have a much lower error rate than long-read sequencing data; this is due to the higher copy-number of reads that can be produced for a given region of the genome.

Long-read sequencing data offers several advantages over short-read data. Large-scale SVs and long stretches of repetitive DNA are difficult to map using short-read data; unless these regions can be captured on a single read, they can be impossible to definitively map and identify. SVs can significantly impact the phenotype of an organism (Field and Blackman 2003; Mathers et al. 2017), and hint at the evolutionary trajectory of an organism by providing evidence for selection on certain traits (Yue et al. 2017). Long, repetitive stretches of DNA have been implicated in aphid pesticide resistance (Liu et al. 2001), and are an important tool in population-level genetic analyses (Sunnucks et al. 1996; Simon et al. 1999). Historically, these repetitive regions have been poorly mapped, even in model organisms, accounting for many of the gaps in sequenced genomes (De Bustos et al. 2016). All *M. persicae* genome assemblies produced thus far have relied on Illumina short-read sequencing data (Mathers et al. 2017), and as a result little is known about the structure and abundance of repetitive regions in the *M. persicae* genome.

By producing a new *M. persicae* draft genome assembly from PacBio SMRT long-reads and by incorporating existing short-read data, I aim to provide an assembly that takes advantage of both sequencing modes, and thus will cover a higher proportion of the *M. persicae* genome. It is my hope that this new assembly will function as a tool that will foster future discoveries in aphid biology and help advance aphid pest management.

Table 1*:* List of currently available aphid genome assemblies, associated NCBI BioProject Accessions, and publications with details on the assembly process for each genome.

Aphid	Accession	Reference
Black cherry aphid, *Myzus cerasi*	NCBI PRJEB24287	Thorpe et al. 2018
Green peach aphid, *Myzus persicae* clone G006	NCBI PRJNA397782	N/A
M. persicae clone G006	ENA PRJNA319804	(Mathers et al. 2017)
M. persicae clone O	ENA PRJEB11304	(Mathers et al. 2017)
Pea aphid, *Acyrthosiphon pisum,* strain LSR1	NCBI PRJNA13657	International Aphid Genomics Consortium 2010
A. pisum, isolate AL4f	NCBI PRJNA547584	Barberà et al. 2013
Soybean aphid, *Aphis glycines*	NCBI PRJNA551277	Giordano et al. 2020
Cotton aphid, *Aphis gossypii*	NCBI PRJNA431119	Quan et al. 2019
Russian wheat aphid, *Diuraphis noxia*	NCBI PRJNA297165	Burger and Botha 2017
D. noxia, strain RWA2	NCBI PRJNA310344	Nicholson et al. 2015
Grain aphid, *Sitobion miscanthi*	NCBI PRJNA532495	Jiang et al. 2019
Corn leaf aphid, *Rhopalosiphon maidis*	NCBI PRJNA480062	Chen et al. 2019
Cherry-oat aphid, *Rhopalosiphon padi*	NCBI PRJEB24204	Thorpe et al. 2018

3.2. Genome Assembly

3.2.1. Sample collection

All of the *M. persicae* used in this study were taken from a long-standing colony maintained in the Insectary at the Agriculture and Agri-Food Canada Research and Development Center in Agassiz, BC (clone "Gillespie"[2]). Colony aphids were reared on whole bell pepper plants inside BugDorm tents (BioQuip Products, Rancho Domingues, CA 90220, USA) at 19 °C (± 2 °C) 60% (±5%) humidity, and a light-dark cycle of 16:8 h. To keep colonies at low density, once per week old plants were replaced with new plants, transferring ~20 aphids from each old plant to a new plant.

Because all aphids used in this study originated from a single clonal line, aphids could be collected *en mass* while maintaining a genetically homogeneous sample. A group of ~800 aphids (1.5 mg of whole aphids of mixed age) was collected from a pepper plant and flash-frozen in liquid nitrogen. This sample was shipped on dry ice to the Arizona Genomics Institute for processing and sequencing. It is important to note that, due to the bulk nature of this sample, our assembly may contain reads from several variant karyotypes, if there are any present in our aphid colony.

3.2.2. PacBio sequencing

The sample aphids, frozen in liquid nitrogen and kept at -80 °C, were ground to a fine powder in a frozen mortar under liquid nitrogen. High molecular weight DNA was extracted from this powder using a standard sodium dodecyl sulfate (SDS) extraction protocol (Sambrook et al. 2000). The quality of extracted DNA was tested using pulse-field electrophoresis and Qbit spectrophotometry. A genomic DNA library was constructed and sequenced on a PacBio Sequel II platform (Pacific Biosciences [PacBio], Menlo Park, CA 94025, USA) on a single SMRT cell. These procedures produced 6.21 M reads with a read N50 of 19.7 kbp. Based on the estimated genome

[2] Named for Dr. Dave Gillespie, a retired AAFC Scientist who spent his career working to promote and advance the use of biological controls in BC agriculture. Dr. Gillespie made the original collection and maintained the colony at AAFC Agassiz for nearly two decades.

size of 350 Mbp for *M. persicae* (Mathers et al. 2017), this sequencing run produced 281× coverage of the genome.

3.2.3. Computing resources

All analyses were performed on the Cedar computing cluster at SFU Burnaby (https://docs.computecanada.ca/wiki/Cedar). Cedar is a composite cluster of over 58,000 central processing units (CPUs) and 584 graphics processing units (GPUs) run on a Simple Linux Utility for Resource Management (Slurm) grid. Programs that were not available globally on the cluster were installed locally using Bioconda (Grüning et al. 2018).

3.2.4. Genome assembly

I produced three *de novo* draft assemblies from raw subreads using a combination of Canu 1.9 (RRID: SCR_015880; Koren et al. 2017) and wtdbg2 2.5 (RRID:SCR_017225; Ruan and Li 2020). I then validated these candidate assemblies in order to choose the most complete and representative assembly for annotation. The specific parameters I used for this assembly software and all other programs discussed below are included in Appendix B.

Canu is a *de novo* assembler designed to accommodate PacBio reads. Canu uses a three-step process: (1) All subreads are overlapped and a set of consensus ("corrected") reads are generated based on overlap scores; (2) low-quality reads and any adapter sequences left over from the sequencing process are trimmed; and (3) Canu generates a draft assembly using the longest 40× reads from the remaining set of corrected, trimmed reads (Koren et al. 2017). Using Canu, I produced a 528 Mbp draft assembly with 3055 assembled contigs. I polished this draft assembly twice using Arrow, a function of the GCpp tool from SMRTLink Tools 8.0. This assembly is 1.5x the estimated genome size for *M. persicae*. This may be due to un-collapsed haplotigs or unresolved SVs within the read set – Canu separates regions that are >3% diverged (Koren et al. 2017).

Wtdbg2 is a *de novo* assembler for long, noisy reads that uses a two-step process. First, raw reads are assembled without a correction step into a fuzzy De Bruijn

graph[3]. A consensus assembly is then built from this output (Ruan and Li 2020). Using wtdbg2, I produced a 376 Mbp draft assembly with 1344 contigs. I polished this draft assembly once using Arrow.

In order to compensate for the lack of correction in the wtdbg2 workflow, I produced a third assembly using a hybrid approach, where I first corrected and trimmed raw subreads using Canu, then produced a consensus assembly of this output using wtdbg2. This assembly is 374 Mbp with 1151 contigs.

3.2.5. Assembly validation

Candidate assemblies were validated using BUSCO v4 (RRID:SCR_015008; Seppey et al. 2019), with the hemiptera_odb10 BUSCO database and the "pea aphid" option for AUGUSTUS training. Assembly quality was checked using QUAST v5.0.2 (QUAST, RRID:SCR_001228; Mikheenko et al. 2018) with the existing *M. persicae* clone G0061.0 assembly (NCBI PRJNA397782) as a reference (Appendix C). Based on the results of this validation, I chose the hybrid Canu-wtdbg2 assembly for scaffolding and gene prediction. This assembly was deemed the best of the three candidate assemblies because it has the highest percentage of base pair matches to the reference. All three candidate assemblies had similar N50 values (1.27-1.31 Mbp) and number of identified BUSCOs (99.0-99.6%). Table 3 contains summary statistics for the assembly.

3.2.6. Removal of contaminant sequences

Prior to scaffolding, I used a combination of NCBI BLASTn and Blobtools (RRID:SCR_017618; Laetsch et al. 2017) to identify potential microbial symbionts and contamination in my draft assembly. First, I ran the assembly against the NCBI nt database using the BLASTn command line tool. This generated a list of 1022 hits with associated accession numbers. I then used Blobtools to visualize the taxonomic distribution of these hits. Based on these results, I was able to identify a complete *pLeu* plasmid of the obligate aphid symbiont *Buchnera aphidicola* within the assembly and

[3]An *n*-dimensional graph with *m* symbols and m^n vertices, representing all possible sequences of length *n*. Wtdbg2 constructs the graph using 1024 bp segments as vertices, with edges connecting the segments based on their order within the input reads. Thus, m^n is equal to the number of unique 1024 bp lengths within the assembly.

remove the associated contig, along with some contaminants from the sequencing process (Appendix D). *Buchnera aphidicola* was the only microbe represented in my assembly.

3.2.7. Scaffolding

To further refine the assembly, I used the reference-guided scaffolding software RagTag 1.0 (Alonge et al. 2019). RagTag aligns contigs to a reference genome using Minimap2 (Li 2018), a pairwise aligner which is optimized for long-read data. I chose the available *M. persicae* G006 clone assembly (NCBI PRJNA397782) as the reference and validated the resulting scaffolds using BUSCO v4 and QUAST. Scaffolding reduced the number of sequences in the assembly to 970 (including 194 unassigned contigs) and increased the assembly N50 to 1.42 Mbp (Table 2).

Table 2: Summary statistics from QUAST, BUSCO, and AUGUSTUS comparing the Gillespie clone scaffolded assembly to the existing G006 clone assembly (NCBI PRJNA397782).

Feature	Gillespie clone	G006 clone
Genome		
Scaffolds/Contigs	970	4,021
Largest sequence (bp)	6.35 M	2.20 M
Total Length (bp)	373 M	347 M
Scaffold/Contig N50	1.42 Mbp	440 kbp
GC (%)	30.19	30.02
BUSCOs (complete, single-copy)	2,459 (98.0%)	2,482 (98.9%)
Annotation		
Predicted genes	26,114	17,015

3.2.8. Gene prediction

I used the gene prediction software AUGUSTUS 3.3.2 (RRID:SCR_008417; Hoff and Stanke 2019) to produce an annotation for my assembly. AUGUSTUS relies on extrinsic data, such as RNA-Seq, protein, and cDNA reads to build a set of prediction parameters that it can then apply to a target genome. AUGUSTUS provides a set of previously developed prediction parameters for many different species including the pea

aphid, *Acyrthosiphon pisum*. These parameters may be used if extrinsic data is not available for the target genome. Using an iterative training protocol (Hoff and Stanke 2019), I determined that the available pea aphid parameters are the best available parameters for my assembly. AUGUSTUS predicted 26,114 genes in the Gillespie clone scaffolds. Future studies may improve this annotation by applying RNA-Seq or protein data collected from the Gillespie clone.

3.3. Conclusions

Using PacBio long-reads and a custom bioinformatics workflow, I generated a draft genome assembly for a new *M. persicae* clone. This assembly is 26 Mbp (7%) longer than the existing G006 clone reference (NCBI PRJNA397782), with fewer gaps and more predicted genes. Further analysis is needed to fully explore the new features of this assembly. For example, the integration of transcriptomic and protein reads could refine gene annotation, and optical mapping data could be used to further increase scaffold size and reduce gaps, allowing for a more refined analysis of genome architecture and structural variants.

Scaffolding with G006 clone data reduced the number of sequences in the assembly, and increased the length of individual sequences. This offers an example of the complimentary nature of short- and long-read data sets – the scaffold regions spanning the assembled contigs represent regions of the *M. persicae* genome that could not be fully assembled from long-read data alone. It is possible that the coverage of these regions by the SMRT sequencing was too low, and that they were eliminated from the assembly.

A more complete genome assembly, with fewer gaps and longer scaffolds, generally provides a more complete construction of repetitive regions and structural variants within a genome (Yue et al. 2017). In the case of *M. persicae*, a more detailed understanding of genome architecture could contribute to a better understanding of pesticide resistance mechanisms (Liu et al., 2001; Field and Blackman 2003), and could set the groundwork for population-level genetic analysis through the identification of new microsatellite regions and polymorphic regions of *M. persicae* chromosomes (e.g., Sunnucks et al. 1996; Liu et al. 1999; Simon et al. 2001; Yue et al. 2017).

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Appendix A. Life history information of the AAFC Agassiz laboratory colony of *Myzus persicae*

Before designing the protocol for the experiment described in Chapter 2, I measured various aspects of the asexual life cycle of our *M. persicae* colony. These measurements represent the average values collected from 15 aphids, selected from the lab colony as first instar nymphs, transferred to Styrofoam cup chambers on single pepper leaves, and reared until they died. Two of the aphids died before reaching adulthood, and are not included in the reproductive analyses.

Table A1: Number of reproductive days (n=13)

Mean	SD	Upper 95% CI	Lower 95% CI
17.1	5.6	20.1	14.1

Table A2: Total offspring per adult (n=13)

Mean	SD	Upper 95% CI	Lower 95% CI
77.7	25.2	91.7	63.7

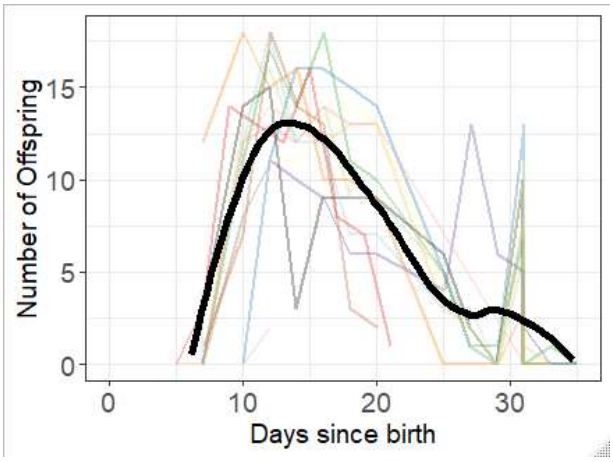

Figure A1: Number of offspring produced by each aphid per 48-h period of lifespan (not including post-reproductive lifespan, which could be as long as 3 weeks). Black line is a LOESS average of al 13 aphids included in this graph.

Appendix B. Specifications for *Myzus persicae* draft genome assembly scripts

Canu 1.9

Identical parameters were used for both the full Canu assembly and to produce corrected, trimmed reads for the hybrid assembly.

```
#SBATCH --time=14-00:00:00
#SBATCH --cpus-per-task=12
#SBATCH --mem=125g
canu -p M-persicae -d M-persicae-canu \
-pacbio-raw <subreads.fasta> \
genomeSize=350m \
correctedErrorRate=0.085 \
gridOptions="--time=08:00:00" \
gridEngineArrayOption="-a ARRAY_JOBS%20" \
ovlMerDistinct=0.975 \
purgeOverlaps=aggressive
```

Wtdbg2 2.5

Identical parameters were used for both the full wtdbg2 assembly and to produce the hybrid consensus assembly using Canu-trimmed, corrected reads.

```
#SBATCH --time=7-00:00:00
#SBATCH --nodes=1
#SBATCH --cpus-per-task=48
#SBATCH --mem=187G
wtdbg2 -x sq -g 350m \
-i <reads.fasta> \
--edge-min 4 --tidy-reads 1000 -L 5000 -t 0 -v \
> wtpoa-cns -t 0 -i ./assembly.ctg.lay.gz
```

SMRTLink Tools 8.0.0 :: Pbmm2

This tool aligns contigs (FASTA) to PacBio subreads (BAM), producing an alignment index and a sorted BAM file of aligned reads. This aligned BAM is needed when running GCpp and BlobTools

```
#SBATCH --time=5:00
#SBATCH --cpus-per-task=48
#SBATCH --mem=187G
pbmm2 index -k 16 \
<contigs.fasta> <contigs.mmi> \
> pbmm2 align <contigs.mmi> \
<subreads.bam>  --sort
```

SMRTLink Tools 8.0.0 :: GCpp

```
#SBATCH --time=2-00:00:00
#SBATCH --cpus-per-task=48
#SBATCH --mem=187G
gcpp -j48 --algorithm=arrow <aligned-reads.bam> \
-r <assembly.fasta> \
```

BUSCO v4

Representative BUSCO script. All BUSCO runs used identical parameters.

```
export AUGUSTUS_CONFIG_PATH=~/augustus-3.3.3/config/
busco -i <scaffolds.fasta> -c 1 -f\
-m geno -l hemiptera_odb10 --augustus_species pea_aphid \
--config ~/busco/config/config.ini
```

QUAST 5.0.2

Representative QUAST script. All QUAST runs used identical parameters.

```
./quast.py --large --fragmented --eukaryote --memory-efficient
<scaffolds.fasta> -r <MPER_G006.fa> -g <MPER_G0061.0.gff>
```

NCBI :: BLASTn 2.10.0

```
#SBATCH --time=5-00:00:00
#SBATCH --nodes=1
#SBATCH -cpus-per-task=48
#SBATCH --mem=187G
export BLASTDB=/yonathan/scratch/ncbi-nt-for-blob/
cd ./ncbi-nt-for-blob
blastn -task megablast -query <scaffolds.fasta> -db ncbi-nt-for-
blob \
-outfmt '6 qseqid sacc bitscore pident length mismatch stitle' \
-max_target_seqs 1 -max_hsps 1 -evalue 1e-10 \
```

BlobTools 1.0.1

BlobTools requires four files as input: 1) A tax file containing FASTA sequence headers, NCBI TaxIds, BLASTn bitscores, and NCBI accession numbers; 2) The target assembly in a FASTA format; 3) A BAM file of the assembly aligned to sequencing reads, with 4) an accompanying MMI index file. I produced the tax file by extracting accession numbers from the output of the BLASTn run detailed above, matching these accession numbers to TaxIds using the NCBI command line tool Entrez Direct, then feeding the resulting TSV into the BlobTools taxify command. The following scripts correspond to the various steps in this process

1. Matching NCBI Accession numbers to TaxIds

```
for acc in `cat <file.tsv>` ; do
  efetch -format docsum -db nuccore -id $acc \
```

```
        | xtract -pattern DocumentSummary -element
AccessionVersion,TaxId \
        >> <tax.txt>;
done
```

2. Producing the tax file

```
./blobtools taxify -f <blobtools.out> -a 0 -b 2 -c 3 \
-m <tax.txt> -s 0 -t 1 \
-o M-persicae-blob-3
```

RagTag 1.0

```
ragtag.py scaffold <reference.fasta> <assembly.fasta> -o
~/ragtag/M-per -u --aligner ~/minimap2/minimap2
```

AUGUSTUS 3.3.2

```
#SBATCH --time=1:00:00
#SBATCH --cpus-per-task=48
#SBATCH --mem=187G
export OMP_NUM_THREADS=$SLURM_CPUS_PER_TASK
java -jar /home/yonathan/GeMoMa/GeMoMa-1.6.4.jar CLI
GeMoMaPipeline threads=48\
outdir=./canu-wtdbg2-gemoma tblastn=false \
GeMoMa.Score=ReAlign AnnotationFinalizer.r=NO o=true \
t=<scaffolds.fasta>
```

Appendix C. Comparison of BUSCO and QUAST results for all three candidate assemblies

Table C1: Summary of BUSCOs identified in a BUSCO v4 analysis of all three candidate assemblies, using the hemiptera_odb10 database and pea aphid for AUGUSTUS training. Total BUSCOs in hemiptera_odb10: 2510

Assembly	Complete BUSCOs	Single Copy	Duplicated	Fragmented	Missing
Canu	2498 (99.6%)	2150 (85.7%)	348 (13.9%)	0	12 (0.4%)
Wtdbg2	2484 (99.0%)	2457 (97.9%)	27 (1.1%)	1 (0.0%)	25 (1.0%)
Canu-wtdbg2	2496 (99.4%)	2460 (98.0%)	36 (1.4%)	1 (0.0%)	13 (0.6%)
RagTag	2495 (99.4%)	2459 (98.0%)	36 (1.4%)	2 (0.1%)	13 (0.6%)

Table C2: Summary of QUAST 5.0.2 results for all three candidate assemblies and RagTag-generated scaffolds, using the available M. persicae clone G0061 assembly as a reference. The majority (8700/9179) of misassemblies reported in the Canu-wtdbg2 assembly are translocations.

Assembly	Number of sequences	Largest contig (bp)	N50 (bp)	NG50 (bp)	L50 (bp)	Misassembled contigs	Genome fraction	Duplication ratio
Canu	3055	16.6M	1.29M	2.37M	90	1384	0.972	1.32
Wtdbg2	1344	4.77M	1.31M	1.51M	82	757	0.955	1.06
Canu-wtdbg2	1151	6.35M	1.27M	1.45M	82	836	0.960	1.06
RagTag Scaffolds	970	6.35M	1.42M	1.58M	78	754	0.959	1.06

Appendix D. Contaminants identified with BLASTn and BlobTools

Table D1: NCBI (National Center for Biotechnology Information) accession numbers associated to contigs in the candidate assembly that belong to microbial or other non-aphid genomes, as identified through an NCBI BLASTn search.

NCBI Accession	Identity	E-value	Organism	Likely Source
CP002698.1	100%	0.0	*Buchnera aphidicola*	Symbiont
AK337409.1	80.91%	8e-56	*Lotus japonicus*	Contaminant
AK337409.1	86.98%	3e-57	*Lotus japonicus*	Contaminant
XM_023874457.1	98.74%	0.0	*Lactuca sativa*	Contaminant
XM_023890820.1	97.06%	0.0	*Lactuca sativa*	Contaminant
KP332739.1	89.52	0.0	*Chrysanthemum x morifolium*	Contaminant