COMMUNITY GENETIC AND ENVIRONMENTAL EFFECTS IN EXTREMELY PLASTIC AQUATIC AND TERRESTRIAL ARTHROPODS

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4. Abbreviations

Word	**Abbreviation**
Acyrthosiphon pisum	Pea aphid
Chrysoperla camea	Aphid lion/ Lacewing larvae
Community Genetics	CGs
Genetically modified	GM
Indirect ecological effect	IEE
Indirect genetic effect	IGE
Interspecific IGEs	IIGE
Intraguild predation	IGP
Intraguild predators	IGPs
Multilocus sequence-typing	MLST
Plant soil feedback	PSF
Quality value	Q
Red Queen hypothesis	RQH
Thyroid hormones	TH
Transgenerational plasticity	TGP
Transient receptor potential	TPR
trans-β-farnesene	EBF
Within-generational plasticity	WGP

5. General Abstract

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Half of my MPhil used a parasitoid-host system (Pea aphid & *A. ervi*) to understand the complex interactions, created by community genetic (CG) effects and other ecological factors, that drive evolutionary dynamics. We designed an experiment that sought to understand how intraguild predation (IGP) and indirect ecological effects (IEEs) (aphid symbiosis with protective symbiont) affect the outcome of an established indirect genetic effect (IGE). We established a quantitative genetic half-sibling design in the parasitoid wasp *Aphidius ervi* to understand the genotype-specific effects on the phenotype of the pea aphid (*Acyrthosiphon pisum*) in the presence and absence of an intraguild predator (*Chrysoperla carnea*). This work aims to improve the integration of CGs into biological pest and disease control schemes in agro-ecosystems, where pest species are becoming resistant to the conventional chemical control methods and an improved understanding of the wider environmental impacts of chemical controls render them increasingly unsuitable

The experiment utilised two clonal populations of pea aphid, the established lab clone N116 and a local isolate named the 'Quad' clone, that we established from a female we sampled from our university quad. We did this to try and understand the effect of their different secondary symbionts, identified using 16s rRNA sequencing, on the outcome of the interaction between the parasitoid and host. Moreover, we wanted to try and understand the differences in wasp virulence that we found in our aphid clones.

Our analysis showed that the main predictor of wasp virulence was the immunity factor in our aphid clones and that aphid behaviour was significantly influenced by a sire effect and an interspecific IGE (IIGE) effect depending on the context of the interaction. We also identified several secondary symbionts in our aphid clones, most notably the presence of three known defensive symbionts, *Hamiltonella defensa, Fukatsuia symbiotica* and *Serratia symbiotica,* in the N116 clone and the presence of *Serratia symbiotica* in the Quad aphid clone.

The other half of my work, with *Daphnia magna*, sought to explain how changes to the abiotic factors of aquatic environments affect the various behavioural and developmental plastic responses of this keystone species; an understudied area considering the scale of natural and anthropogenic changes faced by ecosystems all around the globe. The types of stress faced by aquatic organisms are multifaceted, the salinization, acidification, light and chemical pollution and increases in temperature now represent real threats to biodiversity across the globe. Moreover, whilst we have a good understanding of the consequences of these when they occur in isolation, we do not yet fully understand the ramifications of the more complex and realistic scenario of these stressors occurring together. The ability of *Daphnia* to survive and reproduce, long term, in environments exposed to increases in salinity and acidity was tested in conjunction with exposure to constant light (e.g. light pollution) and constant darkness (e.g. eutrophic environments with low light incidence).

A laboratory raised clonal population of *Daphnia magna* was exposed to various combinations of these stressors over 30 days to investigate the impact they had on the life history traits of our populations. After 10 days under 24-hour light, and combinations of other stressors, and despite tangible increases in population size of some treatment groups, the normal reproduction of our daphnia populations was severely disrupted when compared to the controls. Moreover, after 30 days and across all treatments the reproductive success of the daphnia populations (in 24-hour light) dramatically increased, suggesting a plastic response in daphnia tolerance to the treatment conditions. However, in the absence of any light, high mortality occurred across all treatments indicating that it had a much greater negative impact than constant exposure to light. The age structure of the populations, across all combinations of stress and in the absence of a light cycle, varied significantly suggesting that the life history responses of the populations were context specific. Our findings further our understanding of the ecology of a keystone aquatic crustacean under complex abiotic environmental stress and the ability of aquatic organisms to adapt to the novel environments created by anthropogenic effects.

6. Declaration

i. No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institutes of learning.

ii. However, parts of this work are being written with the intention of my lab submitting them for consideration of publication in international peer-reviewed journals.

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8. Preface

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Conferences where this work was presented

1. Oral presentation at the 2017 Congress of the European Society for Evolutionary Biology, Symposium of eco-evolutionary dynamics (August 2017)

Publications during MPhil

1. Khudr, M.S.*, Purkiss, S.A.*, de Sampaio Kalkuhl, A. and Hager, R., (2018). Novel resilience in response to revitalisation after exposure to lethal salinity causes differential reproductive success in an extremely plastic organism. *PeerJ*, *6*, p.e5277. *Equal contribution

2. Khudr, M.S.*, Purkiss, S.A.* and Hager, R. (2018). Indirect ecological effects interact with community genetic effects in a host–parasite system and dramatically reduce parasite burden. *Proceedings of the Royal Society B: Biological Sciences*, 285(1886), p.20180891. *Equal contribution

3. Khudr, M. S., Fliegner, L., Buzhdygan, O., Purkiss, S. A. and Wurst, S. (n.d.). Disturbance by companion planting affects prey population dynamics under the influence of host occupation and predation by lacewing. *PeerJ.* **(under revision)**.

4. Purkiss, S. A.*, Khudr, M. S.* and Hager, R. (n.d.). Reproductive and phenotypic plasticity of an important aquatic model organism to multiple abiotic stressors. **(in prep)**. *Equal contribution

5. Purkiss, S. A., Khudr, M. S., Vargas, O. A. and Hager, R. (n.d.). The ecological genetics of a model agro-ecosystem. **(in prep)**.

9. General introduction

9.1 Phenotypic plasticity

Phenotypic plasticity was once considered to be an “embarrassment” (West-Eberhard, 1989, p. 1) and a “nuisance” (Pigliucci, 2005, p. 1) to evolutionary biologists who had no explanation for the phenomenon. It was often cited that environmentally induced polymorphisms were simply mistakes, an inevitable result of having such complex genetic machinery (West-Eberhard, 1989). Evolutionary theory has moved on substantially since the 1960s and so has our understanding of plasticity. The term ‘phenotypic plasticity’ is now most commonly used in association with ideas of developmental plasticity and is defined as the ability of organisms with identical genotypes to develop alternative phenotypes as a response to environmental variation (Bijlsma and Loeschcke, 2013). Moreover, these phenotypic changes include alterations of an organism’s morphology, life history and behaviour (DeWitt and Scheiner, 2004).

The broad definition of plasticity means that the types of traits that constitute “plasticity” are often open for interpretation, as it can be argued that all biological traits are in some way influenced by their environment (DeWitt and Scheiner, 2004). Under the condition that a trait is considered exclusively with regard to its genotype × environment interaction then there is no issue with such a broad definition of plasticity (DeWitt and Scheiner, 2004). Plasticity is omnipresent in life (Murren et al., 2015) and can act as a diversifying factor in evolution that provides opportunities for evolutionary change as it contributes to the development of novel phenotypes (West-Eberhard, 1989). Plasticity can produce both non-adaptive environmentally produced variation and adaptive conditional responses (West-Eberhard, 1989).

Phenotypic plasticity is also considered a trait in its own right as it is contingent on natural selection and other evolutionary processes. The plastic responses to the same environmental change will differ between populations of the same species. The plastic responses of populations differ in their direction and severity; this variation is the result of the genetic control of phenotypic plasticity (West-Eberhard, 1989).

The term plasticity can be used to describe a trait at the population level, where it refers to a statistical measure of how the mean trait value, across a

population's genotypes, changes in response to environmental change (Pigliucci, 2005). When used in reference to an individual, plasticity describes the phenotypic expression of a single genotype across multiple environments, this is known as a 'reaction norm' (Pigliucci, 2005). A reaction norm is a graphical representation of phenotypic plasticity where each plotted value represents a specific value for a phenotypic trait (*e.g.* length, weigh, clutch size, etc.) across multiple environments or treatments (Whitman and Ananthakrishnan, 2009; Woltereck, 1909).

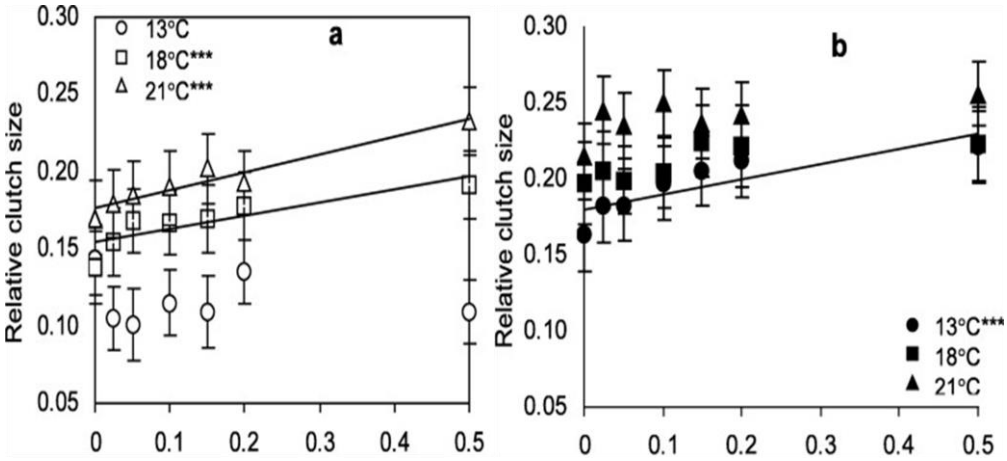


Figure 1: Graphic representation of a reaction norm

Daphnia pulex were raised at three temperatures in both a and b. a represents a group who were fed a low feed level and b a high feed level. Both groups a and b were exposed to varying concentrations of a predatory fish kairomone and its effect on the clutch size in *D. pulex* (plastic trait) is shown. The lines are fitted using a linear regression and were statistically significant. Adapted from (Weetman and Atkinson, 2002).

The variation that results from phenotypic plasticity is measurable and therefore is usually expressed and analysed using an ANOVA (statistical analysis of variance), this is known as variance partitioning. Variance Partitioning is used to try and understand why populations differ in their plasticity and were originally designed to analyse genetic variation between populations (Fisher, 1919). The variance of a phenotypic trait is summarised by the following equation (Whitman and Ananthakrishnan, 2009):

$$V_P = V_G + V_E + V_{GxE} + V_{error}$$

V_P = Total phenotypic variance for a trait

V_G = Genetic variance (explained by genetic differences in a population)

V_E = Environmental variance (explained by differences in the environment)

V_{GxE} = Genotype x environmental interaction

V_{error} = Unexplained variance, including developmental noise, measurement error, etc.

9.1.1 Types of plasticity

Phenotypic plasticity is a crucial mechanism for the survival of many organisms that persist in changeable environments and can be categorised into one of two loosely defined groups: I) developmental plasticity and, II) acclimation (reversible); (Beaman et al., 2016). Both types are found throughout all life stages, juveniles or adult organisms but they occur independently of one another and have separate selective pressures (Beaman et al., 2016). However recent evidence suggests that they are linked, reversible plasticity is controlled by an organism's developmental processes (Beaman et al., 2016).

This link is important as it means that each type of plasticity did not have to evolve separately because of separate selective pressures, instead, it means that the capacity for acclimation will evolve when developmental conditions give rise to a selective advantage (Beaman et al., 2016). This would also give an explanation as to why acclimation is not expressed in the same way, or at all, in generations of the same lineage, thus reducing the cost of maintaining this trait when it is not advantageous. Moreover, acclimation has the capacity to reduce the fitness cost of developmental plasticity, when the conditions that cause the development of an adaptive phenotype change the offspring are left with a trait value that is no longer optimal (Beaman et al., 2016). Acclimation enables the offspring to compensate and reduce the fitness effects of this.

A good example of this can be seen in the freshwater snail *Physa acuta*. Parental exposure to predator cues causes the display various anti-predator defences

(*e.g.* predator avoidance behaviours, crush resistant shell shape, reduced size, etc.) as a result of transgenerational plasticity (TGP); (Luquet and Tariel, 2016). When *P. acuta* is exposed to the cues from predatory cray fish their offspring display increased 'crawling out' behaviour. This behaviour is energetically costly to gastropods that are typically slow and highly philopatric (Luquet and Tariel, 2016). If the predation risk is then removed from the environment there is a mismatch between the strategies of the parents and their offspring and the offspring phenotype is maladapted to its environment (Luquet and Tariel, 2016).

Whilst the morphological changes of the offspring are an example of developmental plasticity, they are commonly less flexible than behavioural traits. For example, the removal of predatory cues from the offspring environment will act to reduce the occurrence of the crawling-out behaviour (an example of within-generational phenotypic plasticity); (Luquet and Tariel, 2016). Interestingly the offspring still display an increase in the behaviour when compared to their parents, thus they cannot completely compensate for the parental environmental effect (Luquet and Tariel, 2016).

It is important to note that adapting to environmental conditions is not always the result of phenotypic plasticity. The random determination of phenotypes, also known as 'diversified bet hedging', is important to organisms that occupy unpredictable environments as it generates diversity (Philippi and Seger, 1989). Diversified bet hedging is present in numerous taxa and is an alternate means for an organism to adapt to its environment (Beaumont et al., 2009). Bet hedging is a stochastic switching of phenotype and does not rely on the direct sensing of an organism's environment, rather it is based on bet hedging (Beaumont et al., 2009). In unpredictable environments, an evolutionary strategy of maintaining random variation, in traits that directly affect fitness, can have a positive impact on a population's fitness in the long term (Beaumont et al., 2009). Bet hedging may improve long-term fitness as it increases the likelihood that a proportion of the population will express a phenotype that is adaptive when environmental conditions change (Beaumont et al., 2009).

9.1.2 Costs of plasticity

There is a large body of research that has sought to understand the costs of plasticity but thus far no widespread costs have been found experimentally. This may be, in part, due to the difficulty of unwinding the cost of a developed phenotype from the cost of plasticity its self (Murren et al., 2015). If there were no constraints to plasticity, then we should see the existence of "perfect plasticity" (DeWitt et al., 1998, p. 78). This is the ability to detect perfect information from the environment and use this to inform various processes that lead to the development of the ideal trait value, at every developmental stage and in every environment with no fitness cost (DeWitt et al., 1998; Murren et al., 2015). To date, research implies that there are indeed constraints on plasticity, Murren et al (2015) postulate that there are two types of constraint. The first is a cost, any loss of fitness associated with the production of a trait via plasticity (when compared with the same trait in an organism that does not display plasticity). The second is a limit, where an organism is unable to develop a trait with an optimal trait value in each environment.

As the cues that influence phenotypic plasticity are not always reliable there is the possibility that an organism may develop a phenotype that is suboptimal because of a mismatch between the developed phenotype and environmental conditions, thus causing a reduction in fitness (Luquet and Tariel, 2016). In many cases, once a phenotype has developed its trait value is fixed. If the environmental conditions that lead to the development of a phenotype (via TGP) change the resulting offspring will possess a phenotype that is not optimal and could suffer a loss in fitness as a result. The cost of developing a phenotype can be further compounded by the metabolic cost of developing a trait. For example, *Daphnia pulex* (de Geer) produces a helmet structure in response to the kairomones of the phantom midge larvae (*Chaoborus* sp.); (Miyakawa et al., 2015). There is also a metabolic cost of maintaining the mechanisms required for the detection of and response to environmental cues, these include the maintenance of sensory apparatus and signalling pathways (DeWitt et al., 1998).

9.1.3 Evolution of plasticity

The benefit of plasticity is clear, it enables organisms to adapt to environmental conditions and maximise their fitness, however, the evolution of plasticity relies on

certain conditions being met. Scheiner (2013) suggests six propositions for the evolution of plasticity: 1) Plasticity depends on the presence of environmental heterogeneity that impacts on the phenotypic expression of traits. Environmental variation can arise as a result of changing environmental conditions or the movement of an organism through various environments. If environmental conditions were relatively stable through time, there would be little selective pressure to produce adaptive morphs. Patterns of environmental change affect the reliability of environmental cues and form a complex relationship with them. 2) The optimal trait value for plastic traits must vary spatially and temporally (space and time). 3) Plastic traits must meet the conditions required for evolution by natural selection. 4) non-optimal plasticity can occur as the environment, at the time a phenotype was produced, did not provide reliable cues ascertaining to the environment at the time of selection. 5) Suboptimal plasticity can result from costs of maintaining and producing a phenotype and the cost of detection of environmental cues. 6) Suboptimal plasticity can also be the result of any developmental limitations on phenotypic plasticity.

Propositions 1-3 are intended to define the minimum requirements for natural selection of phenotypic plasticity (Scheiner, 2013). They are also required for the evolution of any trait via natural selection but with the added condition of environmental heterogeneity. Propositions 4-6 outline the conditions that restrict the evolution of plasticity (4) external factors, 5 & 6) internal factors (Scheiner, 2013). The reliability of cues, in predicting future environmental conditions, is pivotal in the selection for either plasticity or bet hedging (Wong and Ackerly, 2005). When environmental conditions are predictable and the cues associated with them are reliable then models predict plasticity to be favourable under selection and the opposite is true for the evolution of bet hedging (Simons and Johnston, 1997). Moreover, organisms can develop an adaptive strategy that employs a combination of both bet hedging and plasticity, known as adaptive coin-flipping (Furness et al., 2015).

Another key element in understanding when plasticity is selected is phenotype cost, with evolutionary models predicting that the high costs (and limits) of plasticity could act to constrain the evolution of an optimal phenotype. However, these costs, to a larger extent, only exist as a hypothetical variable in models and there is little experimental data to prove their wider existence (Relyea, 2002). Some

of the costs associated with a phenotype can be mitigated by plasticity, as a phenotype is only expressed under certain environmental conditions and thus will only be produced when it is advantageous (Murren et al., 2015).

9.1.4 Mechanisms of plasticity

In order to regulate plasticity, you must first be able to recognise various environmental cues; animals are able to detect many different types of environmental variables during their development and this information can be used to produce an optimal phenotype (McNamara et al., 2016). These developed phenotypes are often essential for adapting to current and expected conditions, the best studied examples of this are adaptive phenotypic plasticity and TGP (West-Eberhard, 2003). Environmental cues vary greatly and can be categorised into one of two groups: I) adult cues, those that are sensed by the adult and affect the development of the next generation, and II) juvenile cues, those that are encountered by the developing organism and directly affect its development (McNamara et al., 2016). Figure 2 summarises the cues that inform an organism's development.

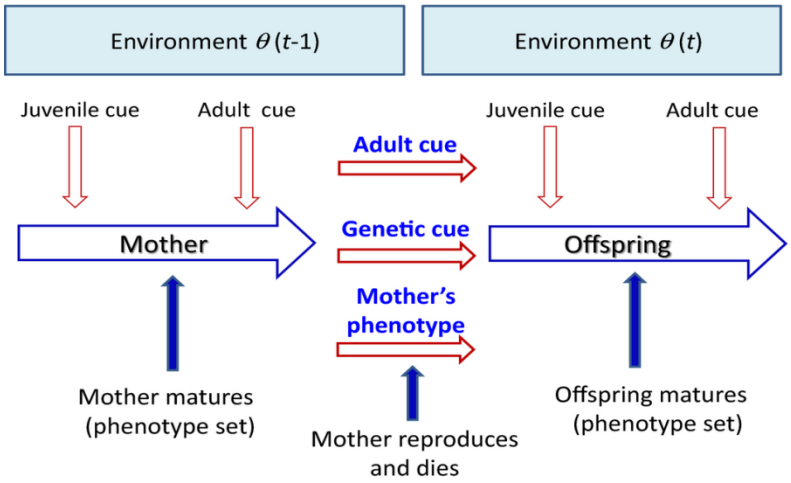


Figure 2: Cues involved in phenotypic plasticity

In the t-1 generation the mother received a juvenile environmental cue which informs its development as it matures, and then her phenotype is fixed. Her fitness is dependent on her phenotype in her current environmental conditions. The cues she receives as an adult do not alter her phenotype but are used to influence her offspring, generation t. The adult cues, in combination with any genetic cues and information about the mother's phenotype, then influence the development of the juveniles. The juvenile cues experienced by t further alter the phenotype of the offspring until they reach an age when the phenotype is fixed, and the cycle continues. Here the adult maternal environmental cues and the offspring's juvenile cues are detection-based cues and the maternal phenotype and genetic cues are selection-based cues. If the phenotype development is affected by environmental cues, the mother's phenotype (cue) then development will combine both detection and selection to influence phenotype. Adapted from (McNamara et al., 2016).

In environments that vary spatially, it has been shown that genetic variation regulates, in part, the ability for an organism to adapt to its local environment and that allele frequency will also vary in association with the spatial variation (Sultan and Spencer, 2002). Moreover, this means that an individual's genotype contains information about its local environment and can be thought of as a genetic cue, that will act in combination with environmental cues and transgenerational effects to inform development (Leimar et al., 2006). In contrast to spatially varying environments, temporally alternating environments are influenced, on a developmental level, by environmental cues and, less often, transgenerational cues.

Animals detect environmental cues in many ways and they include information about multiple environmental conditions such as temperature, light and the presence of a predator. Each cue elicits its own mechanism of detection and they are extremely varied amongst different organisms (Aubin-Horth and Renn, 2009). In many cases a stimulus is required to reach, exceed or drop below a certain level before it causes any change in an organism's phenotype, this is known as "condition autonomous regulative development" (Schmalhausen, 1949) or a developmental switch (Stearns, 1989). Developmental switches are found throughout the animal

kingdom. One example of an environmentally induced switch is displayed in the omnivorous spade-foot toad tadpole (*Scaphiopus multiplicatus*), where the development of a faster developing carnivorous morph is triggered by a high density of its prey (fairy shrimp) and an increase in pond drying rate (Pfennig, 1990).

Drosophila use a small group of warmth-activated anterior cell neurones, located in their brain, that function via the activity of ion channels (Hamada et al., 2008). The receptors form a member of the temperature sensitive transient receptor potential (TRP) family, known as thermoTRPs and are highly responsive to changes in temperature. The TRP channel dTrpA1 acts as a molecular sensor of heat and elicits a heat avoidance response in the *Drosophila* larvae.

Arthropods use a vast array of chemical signals to detect any risk of predation (Chivers and Smith, 1998; Kats and Dill, 1998). Failure to detect a predator can have severe and direct consequences on the fitness of an organism, detection of predators is usually mediated through chemical signals (both direct and indirect) but can also be in the form of visual and mechanical cues (Dicke and Grostal, 2001). Chemical signals are detected through taste, olfaction or a mixture of the two and come in one of two categories: I) Volatiles, that can be detected for a short period of time after production and II) Non-volatile chemicals, these are much longer lived in the medium after production (Dicke and Grostal, 2001). A direct cue is one that is released and detected directly from the predator, for example, chemicals contained within the predator's excretions (Dicke and Grostal, 2001). An indirect cue is one that is mediated via an intermediary, such as alarm signals form an injured or deceased conspecific (pheromone); (Dicke and Grostal, 2001).

The next steps in the regulation of plasticity are the transmission of the detected signal and then the onset of phenotypic change, this is an area of plasticity that has had relatively less attention resulting from a lack of collaboration between ecologists and developmental biologists (Aubin-Horth and Renn, 2009). However, in the systems that have been researched hormones, secondary messengers and signalling cascades have been implicated in this next step (Aubin-Horth and Renn, 2009). Thyroid hormones (TH) T_2 and T_3 regulate the acclimatisation of zebra fish (*Danio rerio*) by altering the transcription and expression of target genes directly (Little et al., 2013). Hormones can also regulate gene expression indirectly by altering the levels of secondary messenger molecules such as cAMP, IP_3, and Ca^{+2} ions intracellularly, thus changing cell state and therefore gene expression (Lema,

2014). The nematode *Caenorhabditis elegans* uses a battery of secondary messenger molecules to regulate all aspects of its life history, including larval development, reproduction and aspects of its social interactions (Bose et al., 2012). Signalling cascades are pathways that utilise a series of proteins to induce a change in one or many cellular functions. The TGF-β and insulin/IGF pathway is crucial in controlling dauer formation in *C. elegans*. Errors in either of these pathways can result in the incorrect formation of a dauer when environmental conditions are favourable or the suppression of dauer formation, even when environmental conditions are harsh (Sommer and Ogawa, 2011). The pathways consist of a series of phosphorylation events that culminate in the regulation of transcription factors and in turn this regulates gene expression causing phenotypic change (Sommer and Ogawa, 2011).

9.1.4.1 Epigenetics and plasticity

Many plastic traits with ecological importance, in both plants and animals, are traditionally considered to be the result of the interaction of multiple genes. More recently there has been huge interest in the ability of epigenetic mechanisms to provide environmental feedback to an organism's genetic machinery and in turn inform the development of the best trait value. The importance of these epigenetic mechanisms in phenotypic plasticity is not well understood. This is largely due to the huge variation found in the epigenetic machinery of all eukaryotes (Forsman, 2015).

Transgenerational epigenetic effects that help to control plasticity are considered to be either 'detection-based' or 'selection-based' (Shea et al., 2011). Selection-based transgenerational epigenetic effects are those that are directly observed and inherited by the next generation and are influenced by environmental cues (Shea et al., 2011). They do not require direct observation by an individual to affect its phenotype, the marker, which affects a phenotype and is under selection, is transmitted through generations and as a result of selection on previous generations, the current individuals express an optimal trait value in their environment (McNamara et al., 2016). Detection-based epigenetic effects are reliant upon mechanisms where the epigenetically controlled phenotype of an individual is dependent on the environmental conditions experienced by that individual's parent (Shea et al., 2011); (also known as adaptive parental effects (Uller, 2008)).

DNA methylation has the capacity to regulate gene expression in many ways (Smith and Meissner, 2013) and has been implicated in the regulation of some plastic

traits. It has long been established that the differential intake of royal jelly in honey bees (*Apis mellifera*) produces alterations in DNA methylation that lead to the development of two castes of bee, the worker bee and the queen (Lyko et al., 2010). The exact loci responsible for the differentiation into each caste of bee remain contested (Kucharski et al., 2015) but the differential consumption of royal jelly causes methylation differences in over 550 genes through the action of DNA methylation enzymes (Lyko et al., 2010).

9.2 Community genetics

The term Community genetics (CGs) was coined by Prof. J. P. Collins of Arizona State University (Neuhauser et al., 2003). It is an area of research that seeks to understand the interaction between genes of one species and the populations of other species within its community, it takes its origins from work by Antonovics (1992) and is an attempt to integrate community ecology with modern evolutionary genetics (Agrawal, 2003; Neuhauser et al., 2003). The understanding that evolution can occur on ecological time scales and that a feedback loop must exist between them is key to the mechanism by which community genetics is able to influence the evolution of communities (Schoener, 2011). Community genetics suggests that heritable genetic variation within one species has consequences beyond the population level and its effects extend community and ecosystem wide. These wider impacts are more notable in dominant species and represent extended phenotypes (Whitham et al., 2003). Community genetics was founded on a recognition that ecological systems involve numerous complex interactions within and between trophic levels and not just the simple pairwise interactions that traditional coevolutionary models sought to explain (Rowntree et al., 2011). More traditionally a genotype is said to give rise to a phenotype that is expressed at the individual and population level, but when a genotype results in an interaction with other species its phenotype can produce community and ecosystem phenotypes (Whitham et al., 2006).

Rowntree et al (2011) state that the function of modern community genetics is to understand the role of genetic variation and the potential for evolution in ecological communities, free from the restrictions of coevolution and without the need to adopt new conceptual paradigms, as envisaged by niche construction theory. Rowntree et al. (2011) also believe that community genetics should continue as

envisaged by Antonovics (1992) but within a more recent frame work set out by Jonson and Stinchcombe (2007) as this will enable community genetics to continue to be relevant.

Though it is argued that community genetics may provide a more context-specific and incoherent understanding of the role of ecology and evolution in shaping ecosystems and ecological interactions than originally predicted (Hersch-Green et al., 2011). The need for a greater understanding about how intraspecific genetic variation, evolution, abiotic and biotic environmental factors influence natural selection, the interaction of species, community composition and ecosystem processes is clear as it will enable a more predictive understanding of the interplay between ecology and evolution (Hersch-Green et al., 2011; Rowntree et al., 2011). Genetic correlations between species are often implicated in the evolutionary outcomes of many community genetic effects and these are defined within a quantitative genetic framework (Rowntree et al., 2011; Wolf et al., 2004).

Given that CGs effects include a number of complex context specific interactions it is often necessary to employ quantitative genetic approaches in order to account for or eradicate the genetic variation of the species interacting with the focal organism (Astles et al., 2005). In our experiment we employ a quantitative genetic half sib design, the details of which are explained in full in section 11.3.2, as this enables us to understand all of the variation in the genotype-by-genotype-by-environment (G×G×E) or genotype-by-indirect ecological effect (G×IEE) interaction our quad-trophic (plant-aphid-parasitoid-intraguild predator) system creates, as the genetic variation is eliminated in the clonal population of aphids.

Community genetics includes a number of indirect mechanisms that influence the interaction of ecological and evolutionary outcomes of our study, indirect genetic effects (IGE), interspecific indirect genetic effects (IIGE) and indirect ecological effects (IEE) that are relevant to our study and are defined in the following sections.

9.2.1 Indirect genetic effects (IGEs)

More traditional principles of Mendelian genetics only consider the effect of a genotype (G) on the development of a phenotype (P). However, an organism's environment and social interactions with con- and hetero- specifics can have a huge effect on their genotype (Wolf, 2003). Moreover, there is increasing evidence that the 'social environment' in which an organism lives and develops can alter the

expression of traits and fitness of an organism (Wolf, 2003). In species that exhibit parental care and those with delayed dispersal (like the pea aphid), the interactions between individuals has the potential to influence many facets of the organism's development (Wolf et al., 1998). Indirect genetic effects (IGEs) theory states that the phenotype of an individual can be influenced by the expression of genes in another conspecific individual, importantly without the involvement of a third individual (Agrawal et al., 2001; Moore et al., 1997; Rowntree et al., 2011; Wolf, 2000; Wolf et al., 1998). The foundations of this theory were outlined in Hamilton's theory of neighbour modulated fitness and contrasted the dogma of 'inclusive fitness' (Hamilton, 1964a). Neighbour modulated fitness refers to the effect of the genes, expressed in one organism, on the fitness of another as a result of their social interaction (Wenseleers et al., 2010).

IGEs are key to understanding how behavioural traits can influence evolution and models suggest that they should be found in a number of social interactions (sexual selection, sexual conflict, the maintenance of dominance hierarchies, and evolution of sociality) and should help to provide a renewed understanding of their origin (Bailey et al., 2017). Moreover, IGEs arise as a direct consequence of social interactions and, despite the growing evidence of the importance of IGEs and their impact on the evolutionary dynamics of traits under selection, they are often overlooked (Ashbrook et al., 2015; Ashbrook and Hager, 2017; Santostefano et al., 2017).

IGEs are being discovered in an increasing number of social interactions as they are universal throughout nature. Moreover, these interactions do not need to be between related individuals or even individuals of the same species. The concept of IGEs has been further developed to include interspecific IGEs (IIGEs). These occur as heterospecific interactions between two individuals, within a community (again without the involvement of a third individual); in other words, they occur when the genotype of a species influences the phenotype of another species (Rowntree et al., 2011; Shuster et al., 2006). These interactions require the traits that govern interspecific interactions to be heritable as this would mean that genetic interactions between species are likely to evolve and could provide a mechanism for genetic selection at the community level (Ashbrook et al., 2015; Shuster et al., 2006).

Our experiment relies on an established IIGE of a parasitoid wasp, *Aphidius ervi* (Haliday), genotype on the phenotype of the pea aphid, *Acyrthosiphon pisum*

(Harris), clones (Khudr et al., 2013). In this context, Khudr et al demonstrated that the genotype of the parasitoid wasp was significant in effecting where the aphid host died following successful parasitoidisation by the wasp. Moreover, parasitoidisation even caused an altruistic adaptive suicide behaviour in the aphids, where they abandon their host plant (Khudr et al., 2013; McAllister et al., 1990). Even though we are beginning to understand the implications of IGEs few studies have considered the effect of other factors on these interactions and rather consider them in a pairwise fashion (TerHorst et al., 2015). We hope to establish the effect of other ecological factors, or indirect ecological effects (IEEs), such as intraguild predation, on the outcome of IGEs.

9.2.2 Indirect ecological effects

An indirect ecological effect (IEE) can be defined as an interaction between species that is altered or mediated by the presence of a third species (Rowntree et al., 2011; Strauss, 1991; Wootton, 1994). As all organisms exist as members of large communities that interact with one another these interactions can have an important impact on the fitness of species within the community that impact both ecological and evolutionary dynamics (TerHorst et al., 2015). The more diverse the community the more common the various indirect effects are as the number of theoretical effects increases exponentially with the addition of each species to a community (Abrams, 1992; TerHorst et al., 2015).

Khudr et al, (2018b) demonstrated the kind of impact that IEEs can have on arthropod communities. The study found that introduction of onion-based chemicals and the microbiome associated with an onion plant into the soil of a new host plant, profoundly affected parasite (aphid) fitness through the action of plant soil feedback (PSF). Moreover, the effect of this IEE differed between genotypes of aphid and between cultivar of host plant and Khudr et al concluded that it was a major predictor of pest damage. Furthermore, there was an interaction of host genotype, parasite genotype and soil inoculation with onion and affected aphid fitness (Khudr et al., 2018b). It is clear that the impact of CG effects and IEEs are highly context specific and more work is required to establish how these interactions can help to understand the way in which a dominant genotype of one species, within a community, can influence the interactions between ecological and evolutionary processes, within and across trophic levels of a community (Rowntree et al., 2011). The importance of a

greater understanding of CG effects has never been greater across many applied areas of biology, none more so than food security and the sustainability of agriculture.

For the last century the dependence of agriculture on synthetic pest control and management has grown massively to meet a growing global demand for food in an ever-increasing population (Carvalho, 2017; Gatehouse et al., 2011), with some estimates placing the cost of global aphid crop damage in the billions of US$ (Loxdale et al., 2017). Moreover, the impact of human activity on natural ecosystems is vastly unsustainable and a need for a more integrated and less environmentally damaging approach to food security is required. The use of pesticides is highly non-specific with only 0.1% of applied pesticides reaching the target organisms, with large proportions acting as an environmental contaminate (Gill and Garg, 2014). The effects of this contamination has wide ranging impacts from biodiversity to human health (Carvalho, 2017). New technologies such as the production of genetically modified (GM) crops offer new opportunities for meeting global food demand and increasing productivity whilst also reducing the impacts of anthropogenic effects on ecosystems (Gatehouse et al., 2011). However, as IEEs can play an important role in the evolution of species, as phenotypic responses to IEEs will change according to the genotypes of interacting species and therefor act as a selective pressure (Astles et al., 2005). Whilst it is thought that the impacts of GM crops have a limited CG effect, it is still important that the community wide impacts of GM crops are established as they could have the potential to cause an unintended cascade of impacts on genetic variation and natural selection across an ecosystem or could even be used to further the ability of biological control agents such as aphid parasitoids. (Gatehouse et al., 2011; Rowntree et al., 2011).

The influence of a predator on the interaction between two other species also represents an IEE; intraguild predation (IGP) is a particularly potent indirect effect. Intraguild predation is a type of predation where a predator also acts in competition with another species at a similar trophic level and is an example of interference competition (Fedriani et al., 2000; Polis et al., 1989). IGP is a three-way interaction found across many taxa and trophic levels and can impact the distribution and abundance of the species as well as their evolution. (Arim and Marquet, 2004; Holt and Polis, 1997; Polis et al., 1989).

In our plant-aphid-parasitoid-predator system, both the aphid's natural enemies (parasitoid wasp and aphid lion) share the aphids as a resource. The aphid acts as both a puparia for the parasitoid juveniles and a prey item for the aphid lion. Importantly the aphid lion consumes both healthy and parasitised aphids and therefor represents an example of IGP. Moreover, the aphid is a clonally replicating organism, allowing us to untangle the IGEs and predator effects (IEEs) independently of genetic variation in the aphid. The IGEs are quantified on aphid polyphenism, behaviour and fitness and we will investigate the changes in parasitoid virulence as a result of intraguild predation. Thus, we will be able to establish how the interaction between IGEs and IEEs affects host trait variation and ultimately shapes complex host parasite interactions.

9.3 Study species

9.3.1 Acyrthosiphon pisum

Aphids express many types of plastic traits when exposed to environmental pressures like predation risk, these include behavioural traits such as adaptive suicide (Khudr et al., 2013) and developmental traits such as size at pupation and clutch size (Henry et al., 2006). Indirect genetic effects (IGEs) occur in a parasitoid-host system when the genotype of the parasitoid influences trait expression in its host. How this relationship is altered by intraguild predators (IGPs), who share the parasitoid host as prey, is unknown. To investigate how IGEs are modified by IGPs we will establish a quantitative genetic design, using the parasitoid wasp *Aphidius ervi,* to investigate the genotype effects of the wasp on the phenotype of the pea aphid (*Acyrthosiphon pisum*) in the presence and absence of an IGP, the aphid lion (*Chrysoperla carnea* (Stephens)).

9.3.1.1 Life history

Aphids are small, phloem feeding, soft-bodied insects that have complex life cycles and highly specialised relationships with their host plant (International Aphid Genomics Consortium, 2010). *Acyrthosiphon pisum*, also known as the pea aphid, is a species of aphid that feeds on various species of *Fabaceae* (legumes). Aphids are a major agricultural pest and have become important biological models for the study of various areas of biology (insect-plant interactions, symbiosis, virus vectoring and

phenotypic plasticity); (International Aphid Genomics Consortium, 2010). The annual crop losses attributed to aphids is estimated to be anywhere between hundreds of millions to billions of dollars worldwide (Blackman and Eastop, 1984; Loxdale et al., 2017; Morrison and Peairs, 1998; Oerke et al., 1994).

The pea aphid feeds at the plant sieve elements where it inserts its mouthpart and injects saliva, containing multiple proteins, that disrupts the plant's defences enabling prolonged extraction of phloem sap (Mutti et al., 2008). The phloem sap contains simple sugars and an unbalanced mixture of amino acids and the pea aphid requires an intracellular mutualistic bacterium, *Buchnera aphidicola*, in order to compensate for this (Moran et al., 1993). The bacteria produce essential amino acids that are absent or rare in phloem sap (Gündüz and Douglas, 2009).

Aphids have evolved a very complex life cycle, outlined in figure 3, that produces individuals with multiple distinct phenotypes, known as clones. Our study uses two of these, the N116 clone and a local clone that we isolated from the quad of the Michael Smith building at the University of Manchester. Their life cycle involves extensive phenotypic plasticity (polyphenism) enabling them to adapt to ecological situations (Blackman and Eastop, 1984; International Aphid Genomics Consortium, 2010).

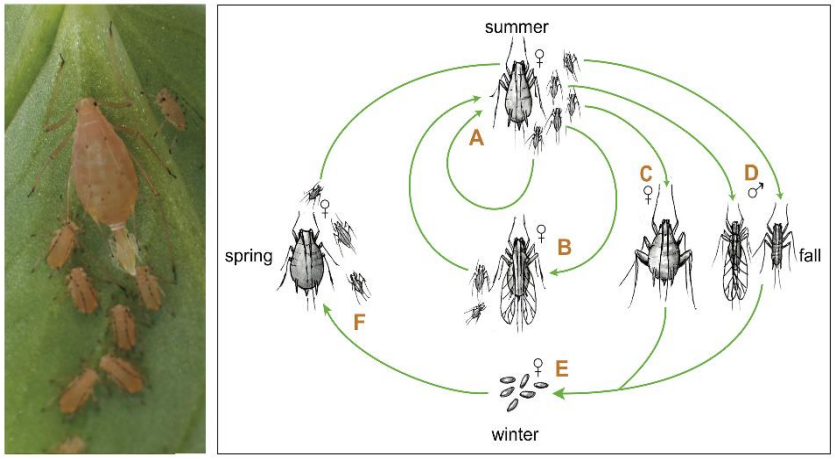


Figure 3: The pea aphid life cycle

During the summer the females reproduce asexually and give birth to live clonal offspring (see photo). During larval development, the offspring undergo four moults and become either wingless (A) or winged (B) asexual reproducing female adults. The development of wing morphs is induced by various stress factors (e.g. overcrowding) and they are more capable of dispersing to other plants. After many cycles of asexual reproduction, the conditions of autumn trigger the aphids to produce unwinged sexual females (C) and wingless and winged males (D). Once mating has occurred the oviparous sexual females produce eggs (E) that lie dormant throughout winter. These eggs hatch to produce wingless asexual females (F). Some populations, especially those that do not experience cold winters, do not produce eggs and have a continuous cycle of asexual reproduction. Adapted from (International Aphid Genomics Consortium, 2010).

The pea aphid genome is 464 Mb in size and is currently being fully assembled and contains many unusual biological features (International Aphid Genomics Consortium, 2010). More than 2000 gene families in the aphid genome have been duplicated and many evolutionarily conserved genes have been deleted (International Aphid Genomics Consortium, 2010). The most notable gene families to be duplicated are those involved in chromatin modification, miRNA synthesis, and sugar transport (International Aphid Genomics Consortium, 2010). There has been deletion of genes that control the IMD immune pathway, selenoprotein utilisation, purine salvage and the entire urea cycle (International Aphid Genomics Consortium, 2010). The genome has also revealed that a small number of genes are bacterial in origin suggesting extensive metabolite exchange between the aphid and its endosymbiotic bacteria (International Aphid Genomics Consortium, 2010).

The epigenome of the pea aphid contains a functional DNA methylation system, small RNA system and an expanded set of chromatin modifying genes and as a result, they are emerging as a key model system for the study of the molecular epigenetics of phenotypic plasticity (Srinivasan and Brisson, 2012). The methylome of the pea aphid is the focus of a large body of research and will give profound insights into the importance of DNA methylation in the control of polyphenism in aphids (Srinivasan and Brisson, 2012).

The pea aphid, like all aphids, is predated upon by a large group of insects including the *Nabis* and *Orius* bugs, *coccinellid* and *carabid* beetles and web-building spiders (Snyder and Ives, 2003). They are also the host for the development of many species of parasitoid wasp larvae, such as *Aphidius ervi* the parasitoid used in our experiment (Snyder and Ives, 2003). Their predators and environmental conditions lead to the development of many plastic traits, *i.e.* morphological, behavioural and life history.

9.3.1.2 Key Traits

9.3.1.2.1 **Morphology**

The development of wing morphs in aphids is produced in response to a wide range of environmental factors including attack by predators and parasitoids (Weisser et al., 1999). Many studies have shown that the presence of predators enhances wing production within a population as an adaptive phenotypic response (Weisser et al.,

1999). The extent to which populations produce this response varies suggesting there is genetic variation for this trait (Weisser et al., 1999). The aphids produce the wing morphs to escape, by flight, from their predators and colonise a new plant. It does not improve their protection against attack but rather enables dispersal when predation risk is high (Weisser et al., 1999).

9.3.1.2.2 **Behaviour**

The pea aphid displays many adaptive behavioural responses to environmental conditions; these include changing their position on the plant and a suicide behaviour. It is well established that the successful parasitoidisation by insect parasitoids can alter the behaviour of an aphid. Aphids infected with the diapausing (suspended development) larvae of *Aphidius nigripes* (Ashmead) move to concealed sites on their host plant before undergoing the mummification process that produces the puparia for larval development (Brodeur and McNeil, 1989; Brodeur and McNeil, 1992). Whilst those successfully parasitised by non-diapausing larvae remove themselves from the aphid colony and the mummies' form on the upper leaves of the plant (Brodeur and McNeil, 1989; Brodeur and McNeil, 1992). Once the aphid is infected with a parasitoid some will abandon the plant in order to increase its exposure to other predators and reduce the chance of the parasitoid emerging and infecting other members of the colony (Khudr et al., 2013).

9.3.1.2.3 **Life history**

Many life history traits of the aphid are also altered by predator interactions, the presence of ladybird larvae, *Adalia bipunctata* (Linnaeus) will induce a reduced fecundity in a population in association with the production of wing morphs (Dixon and Agarwala, 1999). Moreover, one study found that exposure to parasitoid wasps reduces the number and size of the aphids largest embryo (Polaszek, 1986).

9.3.2 *Daphnia manga*

Daphnia are small planktonic crustaceans that are commonly known as 'water flea' and are one of the most commonly used model systems in biological research. *D. magna*, along with *D. pulex*, are two of the most commonly used species of *Daphnia* in a lab setting (Campos et al., 2018). As a keystone species they can be used as an indicator species to assess the health of freshwater ecosystems, they have been

utilized in toxicology and they are widely used as a model system in evolutionary biology, primarily to investigate phenotypic plasticity and adaptive responses to environmental change (Altshuler et al., 2011). *Daphnia*, like the aphid, are a genus of extremely plastic species that express many phenotypic responses to environmental cues, with the *Chaoborus-Daphnia* interaction being one of the most comprehensively studied systems of inducible defences (Oram and Spitze, 2013).

When exposed to the kairomones of the predatory *Chaoborus sp.* larvae *Daphnia* display many forms of phenotypic response to reduce their vulnerability to predation (Parejko and Dodson, 1990) including I) morphological (*e.g.* increase in size, production of 'neck teeth', helmet formation and elongation of their tail spine); (Rozenberg et al., 2015). II) Life history (*e.g.* reduction and delay in fecundity and reduced survivorship); (Havel and Dodson, 1987) III) Behavioural (*e.g.* vertical migration); (Oram and Spitze, 2013).

Daphnia also express phenotypic responses to changes in abiotic environmental factors (*e.g.* levels of pH and salinity), showing similar changes in life history and behaviour to those expressed as a result of biotic factors (Gonçalves et al., 2007; Kring and O'Brien, 1976). However, the importance of epigenetic mechanisms in affecting these changes is poorly understood and the role of the epigenome in mediating phenotypic plasticity has important evolutionary implications. With anthropogenic environmental change becoming ever more important it is crucial to understand how plasticity may affect many conservation efforts.

After extensive pilot work and preliminary investigations using different strains of *D. pulex* and *D. magna*, we decided to use *D. magna*. The *D. magna* populations we had were more stable whereas the D. *pulex* lines we established would regularly die out. The *D. magna* were also able to survive much larger experimental manipulations and for longer periods making it a better fit for our experiments.

9.3.2.1 Life history

Daphnia are surrounded with an uncalcified chitinous carapace and they possess flattened leaf-like appendages that are used to create a water current enabling them to filter out the algae on which they feed. The males are identified by their smaller size, larger antennules, modified post-abdomen and first legs (Ebert, 2005). They inhabit

most fresh and standing water excluding extreme environments and are able to swim throughout their life cycle. Most species of *Daphnia* are pelagic and are predated upon by many fish and insect larvae (Ebert, 2005).

The life cycle of *Daphnia* is similar to aphids (summarised in figure 4) with periods of asexual reproduction followed by stress induced sexual reproduction (Ebert, 2005). Females produce parthenogenetic eggs after every adult moult, these eggs are then stored in a brood chamber, located dorsally in the carapace, where they mature and embryos emerge around 24 hours later (Ebert, 2005). The embryos remain within the brood chamber for three days and undergo further development, when the embryos are ready to be released the mother uses ventral flection of the post-abdomen to force the juvenile *Daphnia* from the brood chamber (Ebert, 2005).

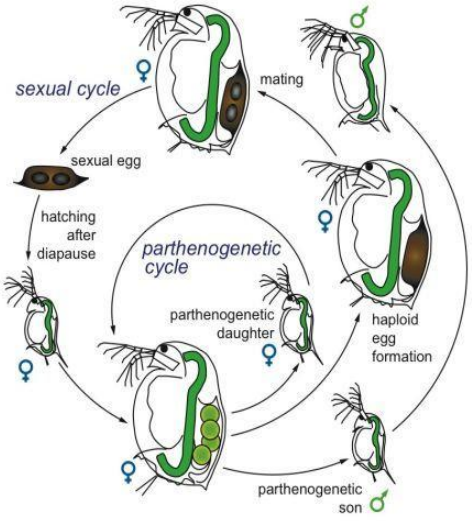


Figure 4: Life cycle of *Daphnia*

During the parthenogenetic (asexual) cycle females produce diploid eggs that develop into asexual daughters. The production of males is controlled by the environment, males develop from asexually produced diploid male eggs. Moreover, females produce haploid eggs in association with male diploid eggs and these require fertilisation by a male in order to develop. The haploid eggs have a different structure to the diploid eggs and are encased in a protective ephippia, and more females emerge after a diapause. Adapted from (Ebert, 2005).

In most species of *Daphnia* the first eggs are released around five to ten days from the emergence of the female, this depends on the quality of the environment, and in poor conditions, it can take longer (Ebert, 2005). The adult female is able to produce a brood of eggs every three to four days until her death. The clutch size varies massively depending on the species with smaller *Daphnia*, like *D. cucullate*, only producing on or two eggs and larger species, such as *D. magna*, producing more than 100 eggs (Ebert, 2005). The life cycle of the *Daphnia*, most notability their ability to maintain parthenogenetically reproducing populations for extended periods,

has enabled the parallel analysis of changes in functional fitness of one genotype across multiple environmental conditions (Campos et al., 2018).

The genome of *D. pulex* is around 200 Mb in length and contains an estimated 30,907 genes (Colbourne et al., 2011). The large number of genes is the result of the many genes undergoing duplication, this results in the formation of tandem gene clusters (Colbourne et al., 2011). The most duplicated regions of the genome are specific to the *Daphnia* lineage and the co-expression of gene families interacting with various metabolic pathways suggests that the maintenance of these duplicated genes is not random (Colbourne et al., 2011). Many of the *Daphnia* specific gene families are devoid of annotation and these regions of the genome are the most responsive to environmental stimuli (Colbourne et al., 2011). Studies of the epigenome have revealed that sex determination, sexual reproduction and the development of many other environmentally controlled phenotypes are mediated through epigenetic mechanisms (Harris et al., 2012). DNA methylation is emerging as the main epigenetic marker that influences the development of predator- and environmentally- induced phenotypes (Harris et al., 2012).

9.3.2.2 Key Traits

9.3.2.2.1 **Morphology**

When adult *D. pulex* is exposed to predatory kairomones, such as those of the predatory larva (*Chaoborus sp*), their offspring produce ‘neck teeth’, a protective helmet structure, and show an increase their general body size (Tollrian, 1995). These phenotypic traits make the *Daphnia sp.* a more difficult prey target for the phantom midge larvae and increase their survival rate (Tollrian, 1995). When exposed to the kairomones of a predatory fish some *Daphnia sp.* respond also by elongating their tail spine; they are only able to do this under high food concentrations suggesting there is a cost relating to the production of this phenotype (Spaak and Boersma, 1997). When exposed to the predatory *Trop cancriformis* or its chemical cues, *D. magna* increases its body size and tail spine length to improve its protection against predation (Rabus and Laforsch, 2011).

9.3.2.2.2 **Behaviour**

Daphnia also displays various behaviourally plastic phenotypes in response to environmental cues. The optimal abiotic conditions (temperature and pH) for

Daphnia feeding occur under the same conditions in which they were raised. Moreover, if these conditions are altered then the feeding rate of the population will fall suggesting they can alter their feeding rate depending on environmental pressures. However, after prolonged exposure (six to eight weeks) to different conditions, the feeding rate can return to its maximal value implying that they are able to alter their feeding strategy in order to adapt to their new environment (Kring and O'Brien, 1976).

The *Daphnia*'s migration through the water column, in response to predator cues, is well established. When exposed fish kairomones *Daphnia* migrate to greater depths to avoid predation, sacrificing the preferential warmth of the surface water (Beklioglu et al., 2008). Conversely, when they are exposed to the chemical cues of the phantom midge larvae they migrate to the surface in order to spatially separate themselves from the predator (Ramcharan et al., 1992).

9.3.2.2.3 **Life history**

Environmental cues inform many of *Daphnia*'s life history stages including clutch size, birth size, the age of first reproduction and growth rate. Many of these traits are altered by biotic agents, such as kairomones, that are released by predators into the water (Pijanowska and Kloc, 2004). One study showed that the stress induced by the presence of phantom midge larvae causes up regulation of heat shock proteins (HSPs) (Pijanowska and Kloc, 2004). The expression of these HSPs, in a clone of *Daphnia*, was associated with an increase in the age at first reproduction, an increase in the size at first reproduction, a reduction in the number of offspring (clutch size) as well as a massive increase in behavioural alertness.

9.4 Hypotheses

9.4.1 Acyrthosiphon pisum

I hypothesise that: 1) The parasitoid wasp, *Aphidius ervi*, will alter the fitness and behaviour of the pea aphid, *Acyrthosiphon pisum*, in a genotype specific way, thus representing an interspecific indirect genetic effect (IIGE). 2) That the addition of the intraguild predator (IGP), *Chrysoperla camea*, will alter the outcome of the IIGE and act to reduce the virulence of the parasitoid in a genotype specific way. 3) Under the threat of the aphid's natural enemies the indirect ecological effect (IEE), imposed by the different bacterial symbionts present in the two clonal lineages of aphid (N116 & Quad), will act to increase aphid fitness and alter the outcome of the IIGE.

9.4.2 Daphnia magna

Here I hypothesise that: 1) The changes to the environmental abiotic factors (salinity, acidity and light cycle) will alter the behaviour and life history traits of our clonal population of *Daphnia magna* and 2) That these changes to *Daphnia magna* behaviour and life history traits will be context specific and will change depending on the exact stress or combinations of stressors that they are exposed to.

10. General Methods

10.1 Aphid

Dr Colin Turnball of Imperial College London provided us with a sample of the N116 genotype pea aphid. From the individuals we received one healthy third instar female was selected and used to establish a colony. The individual aphid female, that was selected from the sample, was reared on *Vicia faba* var minor (Harz), a cultivar of faba bean plant, and maintained at 22-24°C with a photo-period of 16:8 in a lab incubator for many generations. Under these conditions, aphids reproduce through parthenogenesis resulting in a population of genetically identical individuals. The N116 aphid was originally isolated from alfalfa, *Medicago sativa* by Julia Ferrari in Berkshire UK and has been kept under lab conditions for many years (Kanvil et al., 2014).

We also used a second genotype in our experiments, the 'Quad' genotype. This clone was isolated from the quad of the Michael Smith building at the University of Manchester. In order to capture this strain, we left a number of faba bean plants outside, in pots, spread at random throughout the quad. The plants were checked daily for the presence of pea aphids and watered when necessary. After a few weeks, we had noticed large numbers of aphids on the plants and these were then taken into the lab and their morphology was closely inspected to ensure they were the correct species. To establish a genotype from the wild mix we had found we randomly selected and enclosed a single third instar female from this mix into a terrarium sealed with a fine nylon mesh and placed it in the incubator. The selected female was reared on *Vicia faba* var minor and maintained at 22-24°C with a photo-period of 16:8 in a lab incubator for many generations. The resulting population that we established was composed of genetically identical individuals that we then referred to as our 'Quad' clone.

Maintenance of the colonies was carried out when required and they were checked every other day to ensure that the aphids continued to reproduce to their maximum. The plants were watered regularly to ensure the soil was always damp and fertilized with plant food once a week. The large quantities of aphids contained within the terrarium meant that the plants would need to be replaced regularly. The plants were all grown from seed in an isolated chamber under greenhouse lamps to

ensure no contamination with aphids of either genotype and placed individually into pots before they were placed into a terrarium. The terrarium used to rear the aphids were set up as in figure 5. When changing the plants, it is important to ensure that as many aphids were moved onto the new plant as possible. Using a damp paint brush it is easy to lift the aphids off the damaged plant onto the healthy one with as little disruption as possible.

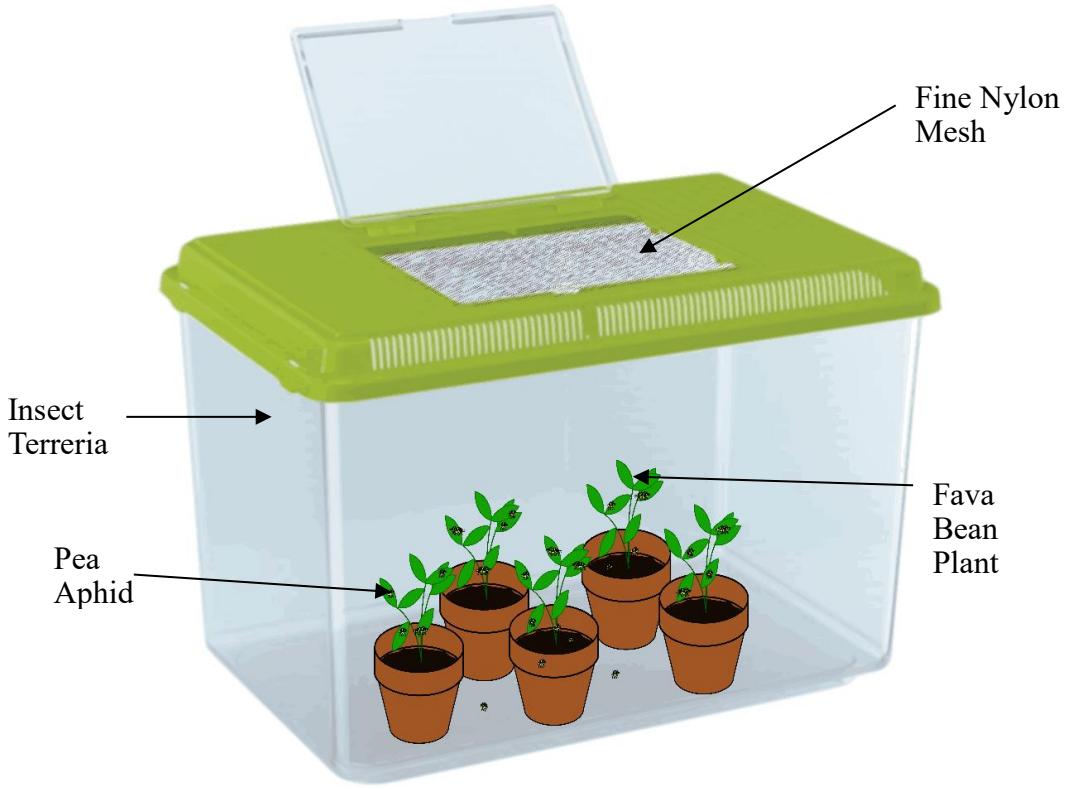


Figure 5: Diagram of aphid terrarium

Each box contained up to five plants in separate pots and a fine non-fray nylon mesh (sourced from Insectopia, UK) was used to cover the entirety of the top of box underneath the lid. This ensured that the aphids were contained within the terrarium but received adequate airflow. Each terrarium was then kept in the previously mentioned standard conditions to ensure parthenogenetic reproduction.

10.1.1 Sterile techniques

When working with multiple aphid genotypes it is important to use a set of sterile techniques to ensure that the aphids of each clone were kept isolated. Each of the aphid genotypes and the new plants were kept in three separate growth chambers to avoid contamination. Only one aphid terrarium was opened at a time and separate lab benches were allocated for working with each of the genotypes. When working with both genotypes it was important to change gloves and brush the sleeves of your lab coat each time you closed a terrarium.

10.2 *Daphnia magna*

A single female of *Daphnia magna* (Straus) was randomly selected from a population, purchased from Sciento©, Manchester UK, and a population of genetically identical individuals was established for use in our experiments. The *Daphnia* were cultured in a growth chamber under fluorescent lights with a light cycle of 16:8 and at a temperature of 23 °C, under these conditions *Daphnia* reproduce parthenogenetically. We kept roughly 50 *Daphnia* within each beaker (approximately 20) and the surplus individuals were either put into another beaker or disposed of. Every 5 days we changed their medium by removing the *Daphnia* with a disposable Pasteur pipette and put them into a new beaker of the medium. When establishing a new *Daphnia* population in the lab it is common for their population to be very unstable whilst they acclimatise to the new conditions. To mitigate this, we left them for more than two months before we began to use them in any experiments.

The *Daphnia* were grown in a medium known as 'Aachener Daphnien Medium' (artificial *Daphnia* medium or ADaM), developed by Klüttgen et al in 1994 and modified by Duneau and Ebert (2012). The Modified ADaM contains only 5% of the Selenium dioxide of the original protocol. The protocol for the modified ADaM we used is summarised in Tables 1 and 2 (all components were sourced from Sigma-Aldrich). The media was made in a 10 L plastic tank with Milli-Q water at least 24 hours prior to use and left in the growth chamber so that the temperature of the medium would acclimatise and ensure that all components had dissolved and mixed properly.

Table 1: Modified ADaM Contents
Adapted from (Ebert, 2013a).

Water (l)	Sea salts (g)	Stock solution A (ml)	Stock solution B (ml)	Stock solution C (ml)
10	3.33	23	22	1

Table 2: Modified ADaM Stock solutions
Adapted from (Ebert, 2013a).

Stock solution	Chemical	Concentration ($g\ L^{-1}$)
A	CaCl2 x 2H2O	117.6
B	NaHCO3	25.2
C	SeO2	0.07

The *Daphnia* were fed every other day with 1ml 'Allinson dried active yeast' (*Saccharomyces cerevisiae*), purchaced at a local store, and 2ml *Scenedesmus quadricauda*. The algae were originally purchased from Sciento ©, Manchester UK and then grown at room temprature on an orbital shaker under constant fluorecent light, in a medium outlined in table 3 (all componants sourced from Sigma-Aldrich) (Ebert, 2013b). The *Daphnia* were fed this way as they performed the best on this diet in our pilot work. Before the algae are put into the *Daphnia* colonies we took an aliquot of the algae culture, usually in a 20ml falcon tube, and then centrifuged it for one minute to remove the algae from suspention, pour away the agal medium and resuspend the algae in ADaM. A new sample of the algal feed was prepared this way every week and the media in the algae culture was also changed weekly. The yeast suspenstion was made every two days by suspending beakers yeast in ADaM at a 0.1g/100ml concentration when the yeast solution was not in use it was stored at 5°C.

Table 3: Algae media components

Medium is produced by mixing 0.115g of TES and then 1ml of stock A-G per litre of deionised water in order. Autoclave medium before inoculating with algae. Adapted from (Ebert, 2013b).

Stock solution	Chemical	Concentration ($g\ L^{-1}$)
A	$CaCl_2 \times 2\ H_20$	36.8
B	$MgSO_4 \times 7\ H_2O$	37.0
C	$NaHCO_3$	12.6
D	$K_2HPO_4 \times 3\ H_2O$	11.4
E	$NaNO_3$	85.0
F	$Na_2SiO_3 \times 5\ H_2O$	21.1
G*	NaEDTA	4.360
	$FeCl_3 \times 6\ H_2O$	3.150
	$CuSO_4 \times 5\ H_2O$	0.010
	$ZnSO_4 \times 7\ H_2O$	0.022
	$CoCl_2 \times 6\ H_2O$	0.010
	$MnCl_2 \times 4\ H_2O$	0.180
	$Na_2MoO_4 \times 2\ H_2O$	0.006
	H_3BO_3	1.000

** Solution G contains among others, all the trace elements*

11. The ecological genetics of a model agro-ecosystem

11.1 Abstract

Interactions between prey and its natural enemies cause complex selection pressures on prey phenotypes. In parasitoid-host systems, indirect genetic effects (IGEs) occur when the parasitoid genotype influences trait expression in its host. However, how intraguild predators, which share the host as prey, modify the influence of IGEs on host traits remains unclear. To answer this question, we established a quantitative genetic design using a parasitoid wasp (*Aphidius ervi*) to investigate its genotype effects on the phenotype of two clonal populations of the pea aphid (*Acyrthosiphon pisum*), in the absence or presence of an intraguild predator (lacewing larvae, *Chrysoperla carnea*). The N116 aphid clone represents a genotype that is highly resistant to the parasitoid and a local isolate the 'Quad' clone that is more susceptible. In this system, both natural enemies share aphids as a provision, where the lacewing consumes both healthy and parasitised aphids (puparia for parasitoid juveniles). Since the pea aphid is a clonal species, we can untangle IGEs and predator effects independent of genetic variation in their prey. Here we quantify IGEs, on aphid polyphenism, behaviour and fitness. We further investigate the changes in parasitoid virulence as a result of indirect ecological effects (IEEs) brought about by the intraguild predator and the presence of defensive secondary symbionts, identified using 16s rRNA sequencing. As such, we establish an understanding of how the interaction between IEEs and IGEs affects host trait variation and ultimately shapes complex host-parasite interactions. We established that the most important predictor of wasp virulence is an immunity factor in the aphid linages and that aphid behaviour was significantly influenced by a sire effect and an IGE effect depending on the context of the interaction. When you compare the two aphid genotypes we found a significant effect of the aphid genotype on both the wasp virulence and the location in which the aphids died following successful parasitoidisation. Moreover, we identified several secondary symbionts in our aphid clones, most notably the presence of three known defensive symbionts, *Hamiltonella defensa, Fukatsuia symbiotica* and *Serratia symbiotica,* in the N116 clone and the presence of *Serratia symbiotica* in the Quad aphid clone. Our findings increase our

understanding of CG effects in agroecosystems and promotes the integration of CGs into to biological pest and disease control schemes in order to relieve reliance on chemical controls.

11.2 Introduction

The fitness of a parasite is dependent on its host and despite a vast range of parasite avoidance and resistance responses, animals are still successfully parasitised (Moore, 2002). Parasites often manipulate their host's behaviour and morphology in order to improve their fitness (Thomas et al., 2011). The behavioural manipulations usually act to either increase the exposure of the host to other stages of their lifecycle, increasing the chance of transmission to the final host, or promote predator avoidance responses in the host, increasing its likelihood of survival (Poulin, 2011). Our work focuses on parasitoidism, a process that represents a mix of both predation and parasitism. Complete parasitoidism occurs when the larvae of the parasitoid consumes and eventually kills its host (Godfray, 1994). The parasitoids are a commonly occurring group of insects, mainly represented by the Hymenoptera (wasps) and the Diptera (flies), that are found in the majority of terrestrial ecosystems (Godfray, 2007). The endoparasitoid in our study, *Aphidius ervi*, is a solitary, generalist and koinobiont wasp native to Europe that was introduced to North America in the 1950s and South America in 1970s, and is the most widely used biological control agent for agricultural pest species of aphid (Ballesteros et al., 2017; Hufbauer et al., 2004).

The co-evolutionary interplay between antagonist species, such as a host and its parasitoid, may drive evolution through a paradigm of reciprocal adaptation and counter-adaptation, producing continuously changing environmental conditions, where there is selection for the development of resistance traits in the host and virulence traits in the parasitoid (Hufbauer, 2001; Janzen, 1980; Thompson, 1994). The strong and intimate interaction between a host and its parasitoid may be influenced by genetic variation, in the traits related to the interaction of the species involved, meeting one of the fundamental criteria for co-evolution in a host-parasitoid system (Henter and Via, 1995). The rate of evolution of resistance traits, to natural enemies, and the infectivity of parasitoids will depend on the level of variation present in the populations and the associated fitness costs of those traits

(Carius et al., 2001; Ferrari et al., 2001). This interplay is an example of the dynamics set out by the Red Queen hypothesis (RQH), which states that interacting species must constantly evolve to maintain their position and is often referred to as an evolutionary "arms race" between species (Lapchin and Guillemaud, 2005). RQ dynamics can result in either reciprocal selective sweeps (*i.e*. the spread of an allele through the host or parasite population followed by the spread of an allele in the other that counteracts it, (Wilfert and Jiggins, 2013) or sustained genotype oscillations, the oscillation of host and parasite genotype frequencies driven by negative frequency-dependent selection (Gandon et al., 2008; Vorburger and Perlman, 2018).

Indirect genetic effects theory has outlined how the genotype of an individual can influence the phenotype of another individual, of the same species (IGE) or of another species (IIGE), but the number of empirical examples remains small (Khudr et al., 2013; Moore et al., 1997; Wolf, 2000). The parasitoid wasp, *A. ervi*, alters aphid behaviour, by influencing where the aphids go to die during wasp larval development. The behavioural modification of the wasp on the aphid is influenced by the genotype of the wasp, and is genotype specific, and therefore represents an IIGE (Khudr et al., 2013).

As previously mentioned and in addition to the behavioural manipulations of a parasite on its host and the counter traits of the host to these manipulations, in a host-parasitoid system there is also an arms race between the resistance of the host, its ability to survive attack by the parasitoid, and the virulence of the parasitoid, its ability to overcome host defences, (Lapchin and Guillemaud, 2005). The resistance of host insect species to their parasitoids is widespread and there is evidence for an endogenous resistance in some insect species, but this is not the only form of resistance (Oliver et al., 2005; Vinson, 1990). In aphid lineages, there is little evidence for resistance via the encapsulation of parasitoid eggs by host haemocytes and is more often mediated by specific microbial symbionts (Kraft et al., 2017; Oliver et al., 2005). Moreover, pea aphid clones show huge variation in their resistance to the parasitoid *A. ervi*, from almost 0% to 100% (Henter and Via, 1995; Martinez et al., 2018), and it has more recently been shown that this variation is often explained by the different linages of the specific protective symbionts found in the different aphid clones, independently of the genetic background of the aphid (Martinez et al., 2018; Oliver and Higashi, 2018; Oliver et al., 2005).

Lineages of the pea aphid, *Acyrthosiphon pisum*, exist in clonally reproducing populations and each of these clones are known to carry several vertically transmitted (secondary) facultative symbionts in addition to its (primary) obligate symbiont *Buchnera aphidicola* (Kraft et al., 2017). The primary symbiont of pea aphids resides in specialised aphid cells, called bacteriocytes, and provides the aphid with nutrients that are lacking in its diet and that it could not otherwise produce (Oliver et al., 2003). The secondary symbionts have been implicated in various functions of the aphids biology, including aiding in host-plant specialisation, thermal tolerance and resistance to parasitoids (Oliver et al., 2005).

Whilst the presence of indirect effects has been demonstrated in the laboratory setting, demonstrating their potentially important role at the community level, there are many aspects of these biotic interactions that complicate our understanding of their effect in the field and on a wider community level. Most experimental examples of indirect effects have demonstrated them as pairwise interactions between the parasitoid and its host. However, in natural ecosystems parasitoids do not operate in a vacuum and are themselves interacting with other species that, for example, share aphid populations as a resource. The aphid lion (*Chrysoperla Carnea* larvae), is another species that is widely utilised as a biological control, and naturally coexists with the parasitoid across its range. The larvae will consume large numbers of pea aphids, including those that have been parasitoidised by *A. ervi* putting these species in competition with each other. This kind of interaction is known as intraguild predation (IGP) and it represents another indirect ecological effect. Intraguild predation occurs when the predator (e.g. aphid lion) also acts in competition with another species at a similar trophic level (e.g. *A. ervi*) (Fedriani et al., 2000; Polis et al., 1989). The effect that IGP may have on the outcome of the IIGE, between the parasitoid and aphid, is unknown and may have important evolutionary consequences and contribute to the pressures that shape the aphid phenotype.

Moreover, a greater understanding of the complex interactions, created by community genetic (CG) effects and other ecological factors, that drive evolutionary dynamics could improve the integration of CGs into biological pest and disease control schemes, and further relieve our dependence on chemical controls in agro-ecosystems. Where pest species are becoming resistant to the conventional chemical control methods and an improved understanding of the wider environmental impacts

of chemical controls render them increasingly unsuitable. In this experiment we have exposed two clonal linages of pea aphid to different genotypes of the parasitoid wasp *A. ervi*, created by the quantitative genetic design, in the presence and absence of intraguild predation in order to first, understand the genotype specific effects of the parasitoid on aphid behaviour and reproductive success, and secondly to explore how the presence of an intraguild predator influences the outcome of the interspecific indirect genetic effect. We also sought to understand the differences in parasitoid virulence between the two aphid populations by identifying their bacterial symbionts and raised the following questions:

1. How does the genotype of the parasitoid wasp *(Aphidius ervi)* impact on phenotypic plasticity of the pea aphid *(Acyrthosiphon pisum)* in terms of fitness and behaviour (IIGE)?

2. Does the addition of an IGP *(Chrysoperla camea)* in combination with the parasitoid wasp alter the outcome of the IIGE?

3. Does IGP alter the parasitoid virulence in a genotype-specific fashion?

4. Under the threat of the aphid's natural enemies, to what extent does the indirect ecological effect (IEE), imposed by the presence of the defensive symbionts in the aphid clones, effect or change the aphid's fitness and behaviour?

11.3 Methods

11.3.1 Study organisms

11.3.1.1 Pea aphid: *Acyrthosiphon pisum*

Two genotypes of pea aphid were selected for the experiment, N116 and our own Quad isolate. The N116 aphid was supplied by Imperial College London and the Quad aphid was established from a pea aphid found in the quad of the University of Manchester's Michael smith building (for full info see 10.1). The aphids were reared on *Vicia faba* var minor (Harz) and maintained at 22-24°C with a photo-period of 16:8. Under these conditions aphid reproduce through parthenogenesis resulting in a

population of genetically identical individuals. Some of the aphid colonies were housed at the University of Manchester Botanical Grounds.

11.3.1.2 The parasitoid wasp: *Aphidius ervi*

We purchased 250 *Aphidius ervi* aphid mummies from Koppert UK Ltd, a generalist species of parasitoid wasp that will infect many species of aphid with its larvae. Immediately upon their arrival, we separated the mummies into multiple 90mm petri dishes (approximately 10 mummies per petri dish), each dish contained a small ball of dental cotton, approximately 20mm in diameter, that was saturated in 10% sucrose solution as sustenance (see figure 6). The petri dishes were kept in a fridge at 10°C to slow the rate of eclosion from the aphid mummies (a.k.a. the wasp puparia). The petri dishes were taken from the fridge hourly and checked for the presence of wasps. If more than one wasp was present in the dish then the gender of the wasp was observed. If all the individuals were of the same sex, then they could be used in the next stage of the experiment. However, if both a male and female were present then they were disposed of to insure the females were virgins. All wasps were then mated and enclosed into their experimental microcosm within two hours of eclosion or they were disposed of.

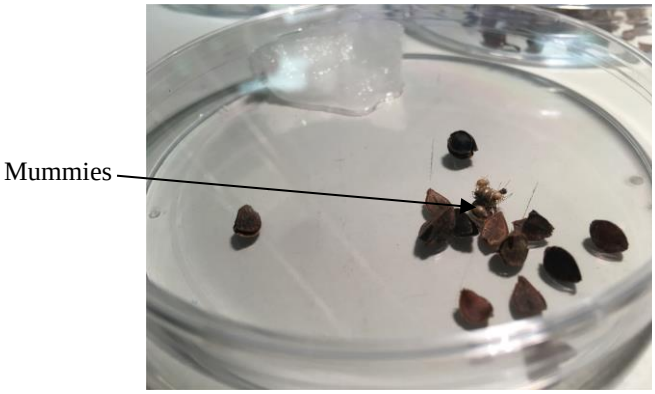


Figure 6: *Aphidius ervi* enclosure
The petri dish contains approximately 10 aphid mummies (wasp puparia), that are delivered in buckwheat husks for protection, and small ball of dental cotton (at the top of the photo) saturated with a 10% sucrose solution.

The life cycle of *Aphidius ervi* can be simplified into several key stages: 1) The female wasp inserts an egg into the body of the aphid. 2) The egg develops into a larva. 3) The larvae consume the aphid from within, eventually killing the aphid. 4) The dead aphid forms a puparia (mummy) for the development of the larvae into an adult. 5) An adult wasp emerges from the rear of the mummy. 6) The newly emerged adult female wasps either a) mate with a male and then infect more aphids or b)

infect aphids without mating. The life cycle of *A. ervi* is haplodiploidy, meaning that males are haploid (1n) and the result of unfertilised eggs and females are diploid (2n) and are the result of fertilised eggs.

11.3.1.3 The intraguild predator: *Chrysoperla carnea* larvae

The intraguild predator in our experiments was the lacewing larvae, *Chrysoperla Carnea* (aphid lion). The larvae were purchased from Ladybird Plant Care, UK in tubes of approximately 300-500 individuals. The tube was emptied into a plastic container, that contained some plant material and aphids, and then kept in a fridge at 5°C until they were needed, this was to slow the rate at which they cannibalised each other. The larvae were used within 48 hours of delivery or they were disposed of and more were ordered. As the wasps take 11 days to emerge after the first stage of the experiments, they were ordered so that they would arrive on day 10 ready to be used in the second stage of the experiment.

11.3.2 Stage 1: wasp mating and establishing the quantitative genetic half-sibling design

During the first phase of the experiment, we mated randomly selected male wasps (sires) with randomly selected female wasps (dams) to establish a quantitative genetic half-sibling design (See section 9.3.2.1). The sires were mated with as many dams as possible within the two-hour period from their eclosion and as a result sire groups ranged from a minimum of three dams with the largest group containing seven dams.

Before the wasps were mated, they were isolated into PCR or microcentrifuge tubes and inspected using a magnifying glass to determine their sex and then labelled. The sex of the wasp is easily identified by observing the abdomen of the wasp; the female's abdomen ends with a pronounced point (ovipositor) and the male's abdomen is more rounded. The wasps were then put into the same tube by opening both tubes and putting them end to end, once both wasps move into the same tube it was sealed with a small piece of foam. The wasps were observed until they

successfully completed copulation (see figure 7).

Figure 7: *Aphidius ervi* in copulation
The figure shows a parasitoid male successfully copulating with a female during the mating step of the experiment. Once mating has completed the wasp separate and we can isolate the individuals.

Once copulation was complete the foam was removed, the tubes were placed end to end and we waited for the wasps to enter separate tubes before closing the lids and labelling the sire with its unique number (S1 – S32) and the dams with the number of the sire they mated and their own unique number in order of mating (*e.g.* S1 D1, S1 D2, S1 D3, S2 D1, etc.) see figure 11 for a detailed breakdown of the mating design. The sire was then introduced to a new female and the process was repeated with as many females as possible, within the two hours from eclosion.

Once mated, the females were placed in their respective microcosms. The microcosms were constructed by removing the ends of a 2-litre bottle and attaching one end to the plant pot and covering the other with a fine nylon mesh. Each microcosm contained a 3-week old broad bean plant that had been infested with 30 third instar pea aphids just before putting the wasp into the enclosure (see figure 8). To release the dam into the microcosm the top section was held in place over the plant (leaving a small gap on one side), the lid of the tube was opened and sealed with the end of a finger and then the tube was passed through the gap onto the soil. Once the wasp was inside the microcosm the top section of the microcosm was secured to the plant pot using 48mm wide polypropylene tape.

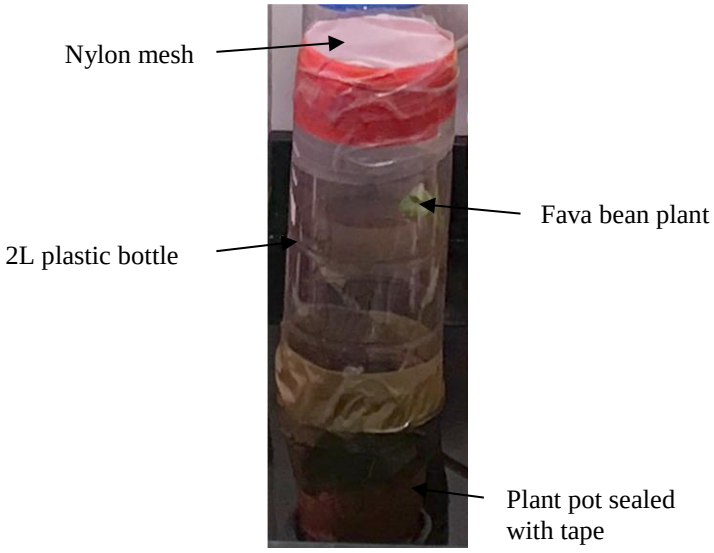


Figure 8: Experimental microcosm
The microcosms were constructed by removing both ends from a 2-litre plastic bottle, attaching a fine nylon mesh to one end and a plant pot to the other. Each microcosm contains a 3-week-old fava bean plant and 30 third instar pea aphids.

The microcosms were spread evenly into large trays, containing a shallow layer of water, in the growth chamber for eleven days (see figure 9). The conditions in the chamber were 22-24°C with a photo-period of 16h light and 8h dark, the water level in the trays was checked and the microcosms position in the trays was randomised every day. On the eleventh day, the microcosms were taken from the growth chamber, opened and all the mummies present were removed from the plant and inner surfaces of the microcosm using a fine damp paint brush. Each mummy was placed into a separate 35mm petri dish that contained a small ball of dental cotton (approximately 10mm in diameter) saturated with 10% sucrose solution and labelled with the sire and dam number. These petri dishes were left at room temperature on the lab bench and left until we observed eclosion. Once the wasps had emerged from the aphid mummy they were examined, and their sex was determined, male wasps were disposed of and the females represented the parasitoid genotypes in the next stage of the experiment. Daughters are selected as only the female wasps deposit eggs into the aphid, and these then develop into puparia for the next generation.

Figure 9: Experimental setup in the growth chamber
The temperature in the growth chamber was kept at 22-24°C with a photo-period of 16:8 under LED lights. The trays under the microcosms contained a small amount of water for the plants and were checked daily when the positions of the microcosms within the trays was randomised.

11.3.2.1 Quantitative genetic half sibling design

Quantitative genetics is the study of the inheritance of traits that are expressed, in a continuous distribution, throughout the phenotypes of segregating populations. These differences give an insight to the inheritance and are a cornerstone of the study of evolution and the application of genetics in the breeding of animals and plants (Falconer and Mackay, 1996).

This experiment employs a nested quantitative genetic paternal half sibling design (half sib design), a simplified structure of one sire group is outlined in figure 6. In order to construct a half sib design randomly chosen males (sires) are mated to a unique group of randomly selected females (dams) (Conner and Hartl, 2004). The resulting offspring is then raised and usually, the phenotypic traits are measured. In our experiment, the phenotypes of the offspring are not measured as we are only using the half sib design to establish differing genotypes of the parasitoid wasp *Aphidius ervi*.

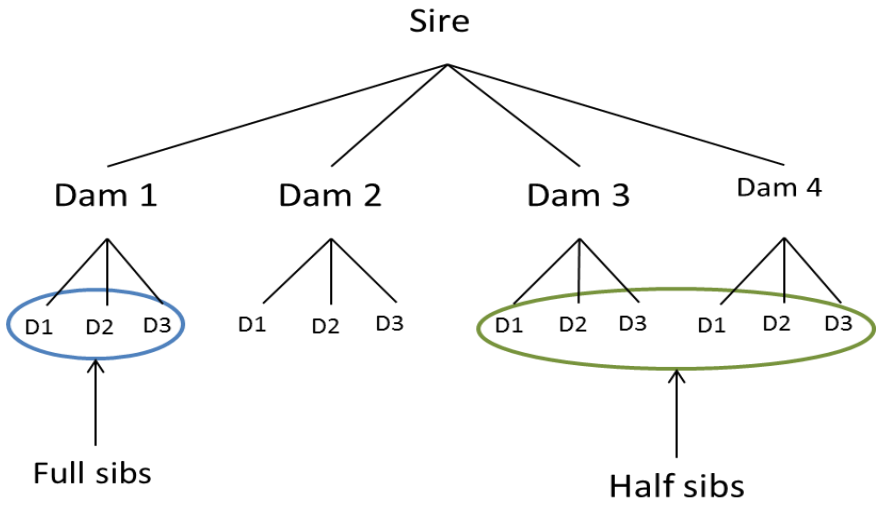


Figure 10: Diagram of a nested paternal half sibling design

Only one sire is shown for clarity and to simplify what would otherwise be a very complex diagram. Many more sire lines may be necessary for the precise estimation of additive variance. In this example the sire is mated to four dams and the offspring from each dam is raised and measured. The daughters of each individual dam represent full siblings and the daughters of all dams are half siblings. 'Sire' refers to a randomly selected male and 'dam' refers to a randomly selected female.

The offspring from each dam represents a full sibling family, as they have the same mother and father (Conner and Hartl, 2004). The offspring, referred to as half siblings, are those that share a father but have different mothers (Conner and Hartl, 2004). 'Nesting' means that different dams were mated to each sire, together these factors produce the hierarchical structure (depicted in figure 10) (Conner and Hartl, 2004). It is critical that the offspring are randomised across the environment they are raised in, as this reduces the chance that the environment they are raised in differs on average across the sibling population (Conner and Hartl, 2004). Moreover, the randomisation of environments ensures that environmental variance is removed from the half sib family groups (Conner and Hartl, 2004). To randomise the environment in our experiment the microcosms were moved around daily to a new randomly selected position within the growth chamber.

The construction of our paternal half sib design means that we created a population of *Aphidius ervi* that possess varying degrees of genetic differences. These genetic differences enabled us to establish if the different genotypes (daughters) of each sire-dam line influence the phenotype expressed in the pea aphid differently, in the presence and absence of an IGP. A full breakdown of the experimental design can be seen in figure 11.

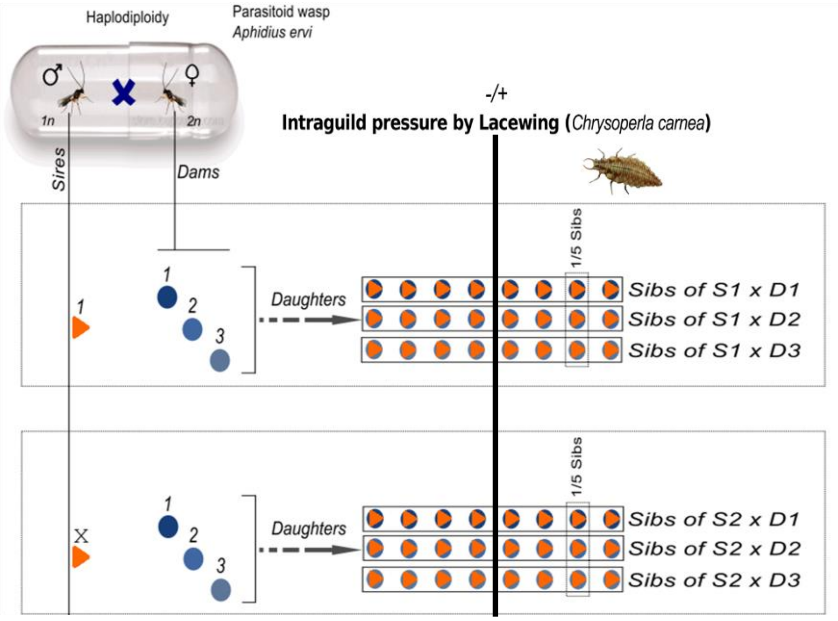


Figure 11: Detailed diagram of experimental design
The diagram shows the breakdown of the full experimental design, with x sires being mated to at least 3 dams. The sire × dam matings give rise to full sibling groups of daughters (horizontally) and half siblings (vertically) in each box. Each group of daughters is then split in half, with one half being exposed to intraguild predation and the other half not.

11.3.3 Stage 2: aphids exposed to wasp genotypes in the presence and absence of IGP

**Please note that at the end of stage 1 we had begun to encounter problems establishing the quantitative genetic design, as a result of the high levels of immunity in the N116 aphid, that will be clarified in section 11.3.4 and this section represents how the next stage of the experiment would have continued had the previous stage progressed as expected.*

As previously mentioned the daughters from each sire-dam line represent a 'genotype' of wasp in our experiment (as in Khudr et al (2013)). The daughters that arose from each of the sire × dam matings were numbered and then split randomly into one of two groups (50:50), 1) with IGP (Y) and 2) without IGP (N). As the wasps emerged, they were carefully caught in a microcentrifuge tube and then placed into a microcosm as in stage 1. If the female was a member of the IGP group, then a lace wing larva was taken from its enclosure on a fine paintbrush and added to the soil of the microcosm a few minutes after the wasp was added.

Once the microcosm set up was complete, they were sealed and placed back into the growth chamber for eleven days at 22-24°C with a photo-period of 16:8. As in stage one, the microcosms were randomised in the chamber and checked to ensure

that they had enough water every day. On the eleventh day, the microcosms were once again removed from the growth chamber, opened and the data was recorded.

We recorded the total number of healthy aphids, the total number of mummies and the distribution of the mummies within the microcosm (on- or off-plant, top 1/3, middle 1/3, bottom 1/3) as shown in figure 12 The healthy aphids in each microcosm were put into a cryogenic tube and frozen at -195 °C, at the University of Manchester liquid nitrogen sample storage facility, for later use.

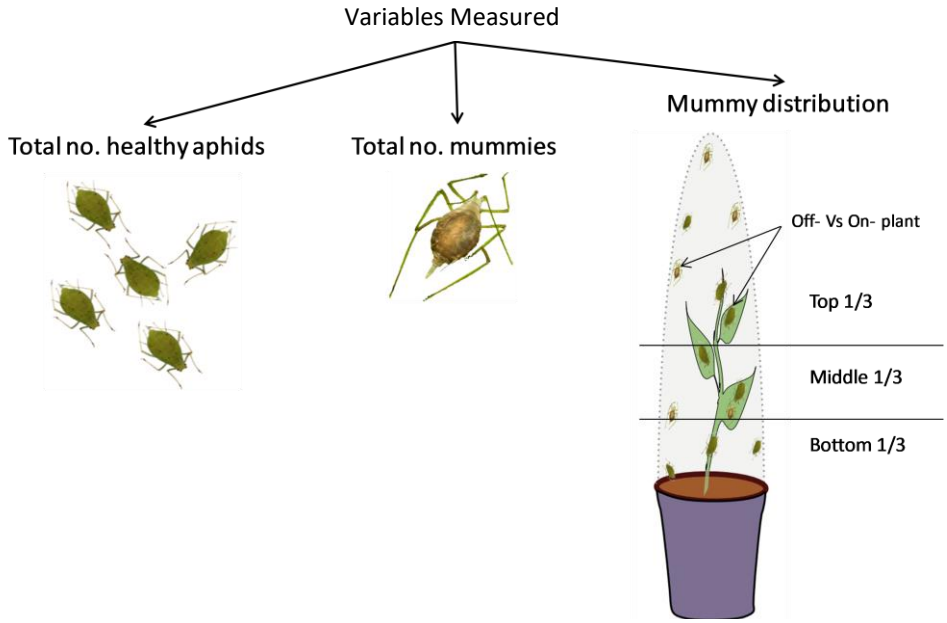


Figure 12: Diagram of measured variables
Once the microcosms where opened after 11 days we first counted the total number of healthy aphids in each microcosm and then the total number of mummies. The distribution of the mummies within the microcosm was measured by the following 5 categories 1) Off-plant. 2) On-plant. If the mummy was 'on plant' we then recorded if it was: 3) top 1/3 of the plant. 4) middle 1/3 of plant. 5) Bottom 1/3 of plant.

11.3.4 Experimental failure and modified exposure to parasitoid

As previously mentioned, at the end of stage 1 it became clear that there was a problem with the experiment. The parasitoid dams were producing very low numbers of mummies with the N116 aphid, with as little as 48% of dams producing at least one mummy. Moreover, of the mummies that were produced many were not successfully developing into adult wasps, with only 30% of dams producing at least 1 offspring and 63% of the mummies isolated successfully developed into adult wasps. Of the 162 parasitoid offspring that were produced across all 19 sire lines (83 dams) 64% were female. Our experiment requires a minimum of two daughters (so

that one is exposed to IGP and the other is not) from at least two dams within each sire group to form a useable family group of full and half siblings and only three of the 19 sire lines we created met this criterion.

When the daughters that were produced were used in the second part of the experiment, they were producing even lower numbers of mummies, with only 22% of the daughters created in the first part of the experiment producing at least one mummy in the second part. As a result, the data that we were able to collect was negligible and we decided to alter the method of the first experimental stage to ensure that the dams were successfully depositing eggs into the aphids in the first stage.

We ordered a new sample of parasitoids, as it was possible that the first batch may have been defective in some way, and established 14 new sire lines by the same method outlined in 11.3.2. In this attempt, before we added the 30 aphids to the microcosm, they were placed in a 90mm petri dish, using a fine damp paint brush, along with some plant material and the mated female was then added to the petri dish and observed to ensure they were successfully parasitising the aphids (see figure 13). After 2 hours in the petri dish, the parasitoid females were recaptured in a microcentrifuge tube, the aphids were then placed onto the plant using a paint brush and left for 30 minutes before the wasp was released into the microcosm, as in the previous attempt. The rest of the experimental design continued as in 9.3.2 and 9.3.3.

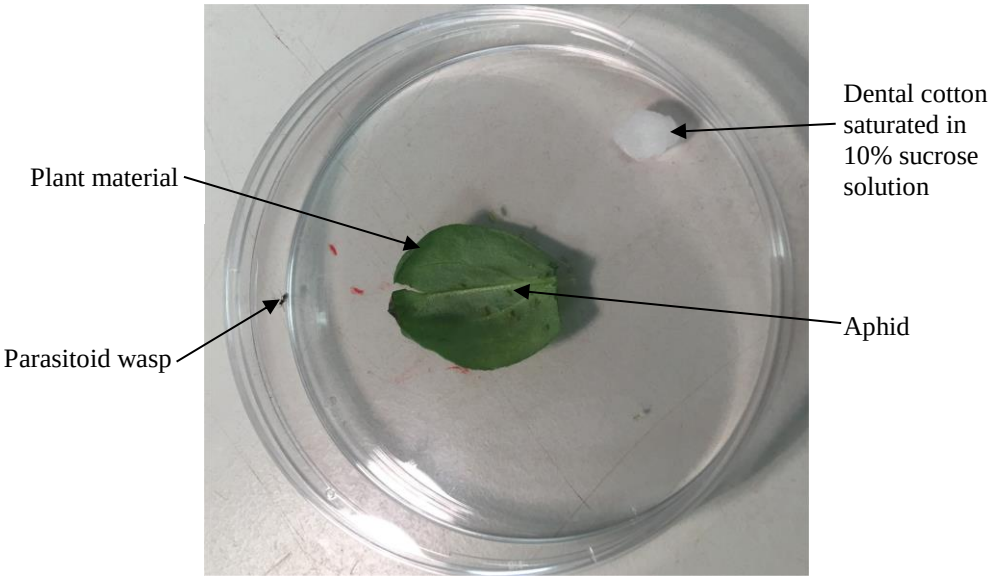


Figure 13: Modified parasitoid exposure
The aphids were exposed to the parasitoid in the petri dish for 2 hours after we witnessed the female successfully deposited an egg into the aphid.

11.3.5 Experimental failure and repeat with the quad genotype aphid

The altered method, using a petri dish to expose the aphids to the parasitoid, was also largely unsuccessful and once again produced very low numbers of useable sire groups. In stage 1 only 32% of dams produced at least one mummy, 27% of dams produced at least one offspring and 70% of the mummies that were isolated successfully developed into adult wasps. Of the 158 offspring that were created across 14 sire lines (71 dams) 54% were female, creating 3 usable family groups.

In stage 2 the low yield of mummies continued with only 14% of the viable females created by the quantitative genetic design producing at least 1 mummy in the second stage of the experiment. At this point, we decided to repeat the original experimental design with another genotype of the pea aphid that we had established, the 'quad' clone, as we suspected that there was a level of immunity in the N116 aphid genotype.

Immunity to parasitoids is a phenomenon that has been observed in some strains of aphid and is provided by the presence of defensive symbionts (Oliver et al., 2003). A symbiont found in many sap-feeding insects, *Hamiltonella defensa* is a known defensive secondary symbiont of the pea aphid that is found sporadically throughout pea aphid lines (Degnan et al., 2009). It provides immunity by stopping the development of *A. ervi* larvae and rescuing the aphid host (Degnan et al., 2009). The level of immunity provided by the different strains of *H. defensa* can vary substantially and the spread of *H. defensa*, in experimental populations, increases rapidly with exposure to parasitoid wasps (Oliver et al., 2009). Although the N116 pea aphid is one of the linages with a known association with *H. defensa* (Kanvil et al., 2014), to my knowledge, the level of immunity in this strain had not been empirically tested.

Repeating the experiment with the quad genotype meant that we were not only able to make comparisons between two the aphid genotypes in terms of their phenotypic responses to parasitoids but compare the bacterial symbionts within the two aphid clones and gain insights into how a symbiont (or IEE) affected the outcome of the IIGE between the parasitoid and its aphid host.

11.3.6 Symbiont Identification

The identification of the bacterial symbionts in the two clone of pea aphid consisted of two parts, 1) the use of diagnostic PCR to confirm the presence or absence of the defensive symbiont *H. defensa* and 2) 16s rRNA gene sequencing for the identification of other symbionts.

11.3.6.1 Diagnostic PCR

To identify the symbionts found in our two aphid clones I followed a previously established protocol that made use of two housekeeping genes (*murE* and *hrpA*) for the identification of *H. defensa* via diagnostic PCR and a comparison of the genetic distance of *H. defensa* strains between aphid linages (McLean and Godfray, 2015). The *H. defensa* specific primers for these genes were taken from a multilocus sequence-typing (MLST) scheme outlined by Henry et al (2013) the details of these primers are outlined in table 4.

Table 4: *hrpA* and *murE* primer information

Gene	Primer names	Stock Conc (nmol)	Tm°	Sequence (5′-3′)
hrpA	hrpA106F	41.8	61.8	AAACCCAATCTGACAAAAATAGG
	hrpA984R	45.1	62.3	TAACTCTTCGGCTTCTGACAAC
murE	murE16F	34.5	59.0	ACTAACGGGAAAACCACTAATAC
	murE936R	30.4	61.6	TTGAGAATGTCAGCGGTAATC

*All Primers were diluted from the stock concentration above to a 1:10 working mix that was used in the PCR reactions

The reagent and equipment used in all the following PCR reactions are as follows (unless otherwise stated):

- Applied Biosystems 2720 thermocycler
- Bioline HyperLadder 1 kb
- Bioline 2x My Taq RedMix
- SafeView Nucleic Acid Stain
- 1% agarose gel
- All primers were sourced from Sigma-Aldrich

All the PCR reactions were set up in the following reaction mix (unless otherwise stated):

- 10 µl Taq RedMix
- 7 µl nuclease free water
- 1 µl forward primer
- 1 µl reverse primer
- 1 µl DNA sample

11.3.6.1.1 **DNA extraction 1**

Before the DNA extraction, I isolated two samples of 10 adult N116 pea aphids from our lab culture and then extracted the DNA using 'Qiagen DNAEasy Blood and Tissue Kit' small insect supplementary protocol (QIAGEN, 2006). As the aphids are soft bodied insects, we altered step 1 of the protocol slightly, rather than freezing them in liquid nitrogen and grinding them up in a pestle and mortar they were frozen at -80 in a microcentrifuge tube order to euthanise the aphids and then homogenised in the tube using a sterile disposable microcentrifuge tube homogenization pestle. The rest of the protocol was followed with no further modifications.

Once the DNA extraction was complete the samples were quantified on a Thermo scientific 2000 spectrophotometer nanodrop to gain an estimate of the DNA concentration and purity (see table 5 for results). The 260/280 ratio is usually used to determine protein contamination of a nucleic acid sample, with around1.8 being generally accepted as pure DNA and around 2.1 being pure RNA, and a low ratio indicates the sample is contaminated with proteins. The 260/230 ratio is an indicator of organic contamination and should be approximately 2.0-2.2, with a ratio lower than 1.8 being considered significant contamination.

Table 5: DNA extraction 1 nanodrop data

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
N116-1	72.7	1.93	1.40
N116-2	64.2	1.89	1.31

11.3.6.1.2 **PCR 1: *H. defensa* specific *murE* and *hrpA* primers**

The first PCR was conducted largely to familiarise myself with the equipment and protocol that we were using. The original protocol made use of a touchdown PCR program, but we did not believe it to be necessary in our case as the two primers that

we used had a similar Tm°. I prepared both N116-1 and N116 two samples into the following conditions and as shown in figure 14 the PCR was unsuccessful, and optimisation of the PCR conditions was required. The PCR reaction components and conditions were:

- 10 µl PCR master mix
- 5 µl nuclease free water
- 1 µl forward primer × 2 (*murE* & *hrpA*)
- 1 µl reverse primer × 2 (*murE* & *hrpA*)
- 1 µl DNA sample
- 94°C 2 mins (94°C 30s, 50°C 50s, 72°C 2 mins) × 25 and a final extension of 72°C 5 mins

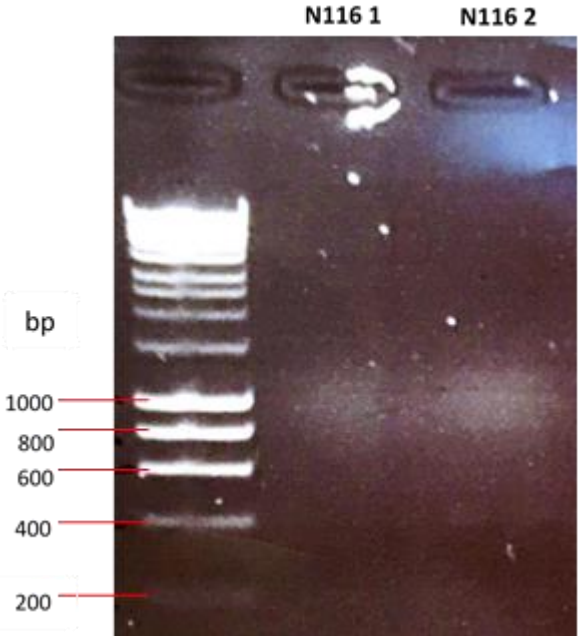


Figure 14: PCR 1
PCR 1 used both DNA samples with both the *murE* and *hrpA* primers A faint hazy band is present in the agarose gel between the 800bp and 1000bp ladder, the expected location but the bands are not clear indicating optimisation of the PCR conditions is required.

11.3.6.1.3 PCR 2: Universal 16s Primers

Following the first PCR, I wanted to ensure that there was enough bacterial DNA in the samples, as a low quantity of DNA can inhibit the production of clear bands. Whilst the nanodrop measurements indicated that there was sufficient DNA in the samples for a PCR reaction, it does not account for the fact that the DNA extraction process results in both aphid and symbiont DNA being extracted together, with the symbiont DNA accounting for a small portion of the overall DNA extracted.

The primers used in this PCR are designed to amplify the 16s region of ribosomal rRNA genes in all eubacteria. These primers, known as 27F and 1492R (Weisburg et al., 1991), amplify DNA between position 27 and 1492 of the bacterial 16s rRNA genes and are numbered according to their position on the *Escherichia coli* rRNA (Frank et al., 2008). The PCR produced clear bands (figure 15) indicating that there was enough bacterial DNA, of sufficient quality, in both N116-1 and N116-2 samples for successful amplification by PCR.

PCR primers & conditions:

- 27f: 5′-AGAGTTTGATCC TGGCTCAG-3′
- 1492r: 3′-ACGGCTACC TTGTTACGACTT-5′
- 95°C 5 mins (95°C 30s, 60°C 30s, 72°C 30s) x 25 and a final extension of 72°C for 7 mins

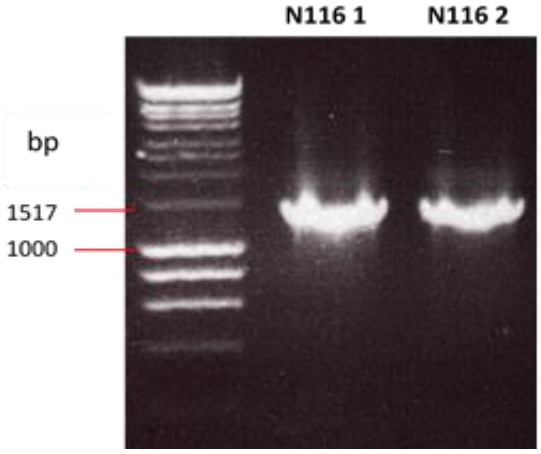


Figure 15: PCR 2
PCR with universal 16s primers, 27f and 1492r, in both DNA samples N116-1 and N116-2. The strong band in the agarose gel indicated that there was enough bacterial DNA in the samples for the identification of bacterial symbionts by diagnostic PCR

11.3.6.1.4 PCR 3: *H. defensa murE* and *hrpA* primer optimisation

The PCR with universal 16s primers had confirmed the presence of bacterial DNA in the samples that I had extracted and as a result, I continued to optimise the conditions. The annealing temperature of the protocol seemed low when considering the manufacturers stated Tm° of the primers, so the PCR was attempted again with the annealing temperature set at either 50°C and at 55°C.

The manufacturers of the My Taq RedMix suggest reducing the extension time as this can reduce the appearance of smearing and non-specific products. As a result, we reduced the extension time to 30 seconds. For this PCR I only used one set of

primers in each reaction, rather than both as in PCR 1, as this would avoid the possibility that the primers are inhibiting each other. Both the *murE* and *hrpA* primers performed better at 55°C, as shown in figure 16, but the bands remained unclear and further optimisation was required. The PCR conditions were as follows:

- 95°C 5 mins (95°C 30s, 50°C OR 55°C 30s, 72°C 30s) x 25 and a final extension of 72°C for 7 mins

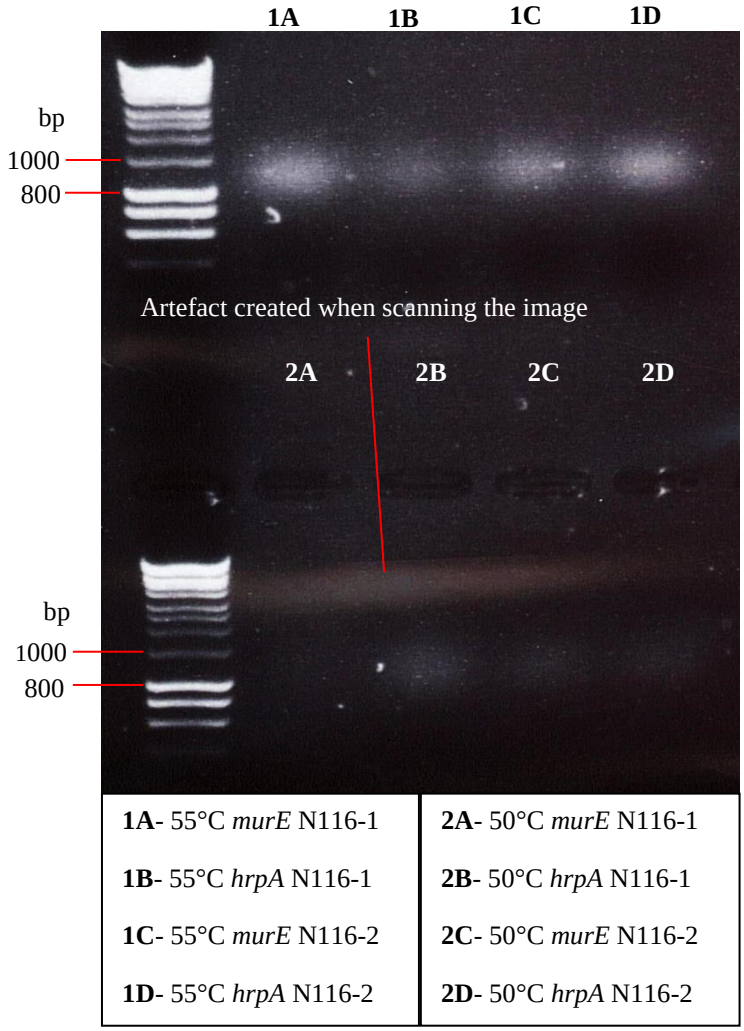


Figure 16: PCR 3
PCR 3 used both the *murE* and *hrpA* primers separately with the N116-1 and N116-2 DNA samples at both 55°C (top row) and 50°C (bottom row). The increased annealing temperature 55 °C improved the outcome of the PCR and a stronger band can be seen in the gel. However, the bands are still distorted, and insufficient thus further optimisation is required.

11.3.6.1.5 PCR 4: *H. defensa murE* and *hrpA* primer optimisation 2

The third PCR showed that the increased annealing temperature to 55°C improved the result of the PCR but it was still not a clear band. In an attempt to increase the clarity of the bands I increased the number of cycles to 40 as this should increase the quantity of the PCR product. However, the increased cycles did not positively affect

the outcome of the PCR and I continued to optimise the conditions of the PCR. The middle lane of the 1% agarose gel (figure 17) was left empty as the well did not form properly. The PCR conditions were as follows:

- 95°C 5 mins, (95°C 30s, 55°C 30s, 72°C 30 sec) x 40 and a final extension of 72°C for 7 mins

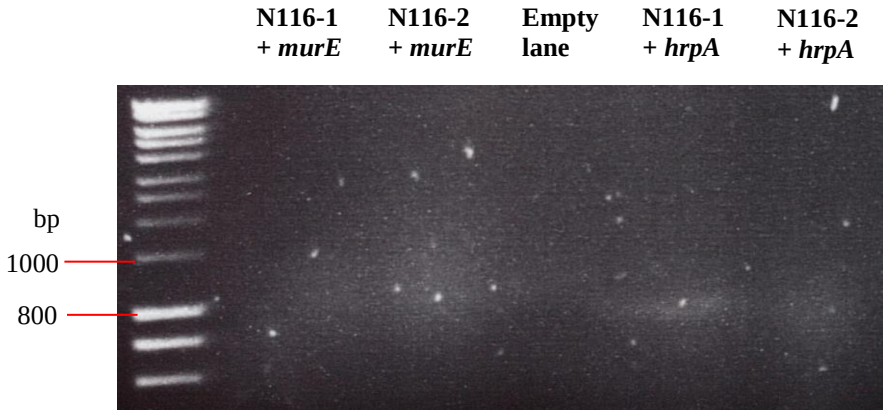


Figure 17: PCR 4
An image of the gel following PCR 4 shows that the increase in the number of cycles from 25 to 40 did not improve the bands appearance in the gel.

11.3.6.1.6 PCR 5: *H. defensa murE* and *hrpA* primer touchdown PCR

As we had little success with a standard PCR program I reverted back to the original touchdown PCR conditions outlined in the protocol given in Henry et al (2013). However, with these conditions, we failed to get any PCR product as shown in figure 18. The touchdown PCR conditions were:

1) "Touchdown" PCR- 94°C 2 mins, 11 Cycles of (94°C 20s, 56°C (Declining 1°C each cycle) 50s, 72°C for 30s)
2) 25 cycles of (94°C 2 mins, 45°C 50s, 72°C 2 mins) and a final extension of 72°C 5 mins

- For this PCR we used a BioRad MJmini gradient thermocycler.

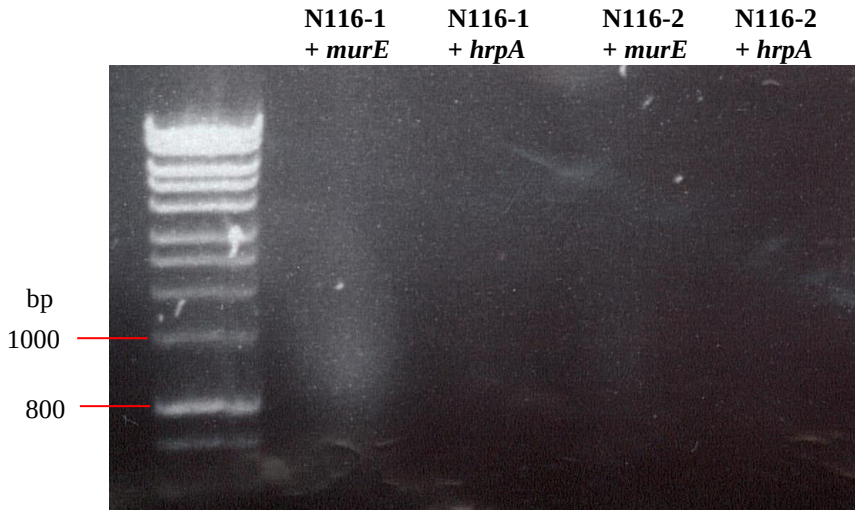
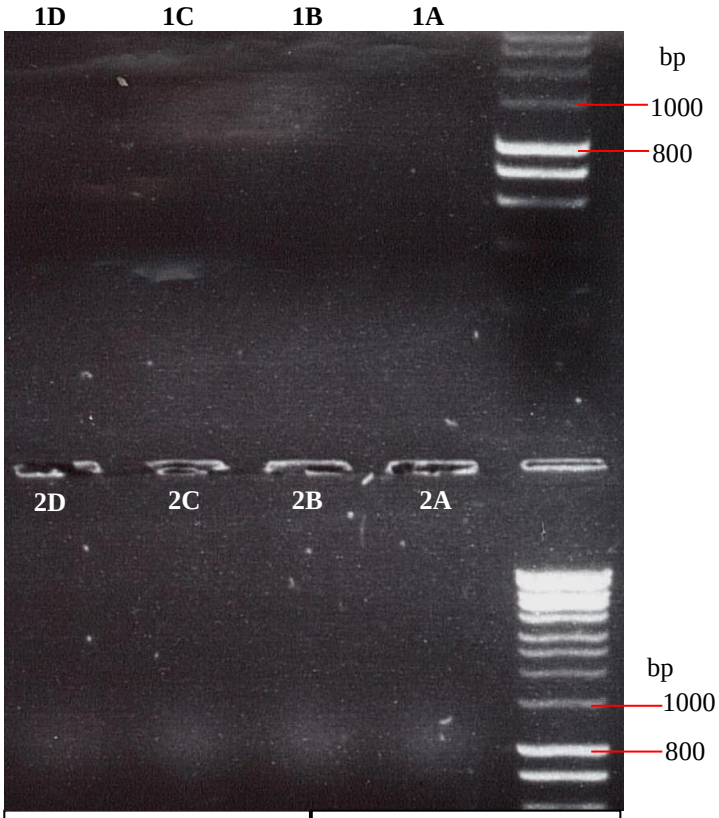


Figure 18: PCR 5
N116-1 and N116-2 DNA samples in a failed touchdown PCR reaction with *murE* and *hrpA* primers.

11.3.6.1.7 PCR 6: *H. defensa murE* and *hrpA* primer optimisation 3

Given the outcome of the touchdown PCR, and after consultation with Dr Jocelyn Glazier and Dr Jon Pittman of the University of Manchester, I did not pursue it any further and instead tried to optimise the conditions of a standard PCR program. As the optimal annealing temperature of a PCR is usually within 2°C of the primer Tm° I increased the annealing temperature to try and improve the outcome of the PCR. In this reaction, the DNA samples a *murE* and *hrpA* primers were run with an annealing temperature of 57°C and 59°C and the results of this are shown below in figure 19. A very faint mark could be seen between the 1000 bp and 800bp ladder in 1A – 1D but these were lost when scanning the image. Moreover, faint bands can be seen in 2A – 2D but they were not as clear as when the annealing temperature was set at 55°C. the conditions for the PCR were:

- 95°C 5 mins (95°C 30s, 59 or 57°C 30s, 72°C 30 sec) x 40 and a final extension of 72°C 7 mins



1A- 59°C *murE* N116-1	2A- 57°C *murE* N116-1
1B- 59°C *hrpA* N116-1	2B- 57°C *hrpA* N116-1
1C- 59°C *murE* N116-2	2C- 57°C *murE* N116-2
1D- 59°C *hrpA* N116-2	2D- 57°C *hrpA* N116-2

Figure 19: PCR 6
Gel following PCR 6 with N116-1 & N116-2 and the *murE* and *hrpA* primers at an annealing temperature of 57°C and 59°C. The bands are very faint and did not represent an improvement over the 55°C annealing temperature of PCR 3, thus optimisation of the PCR conditions continued.

11.3.6.1.8 PCR 7: H. defensa *murE* and *hrpA* primer optimisation 4

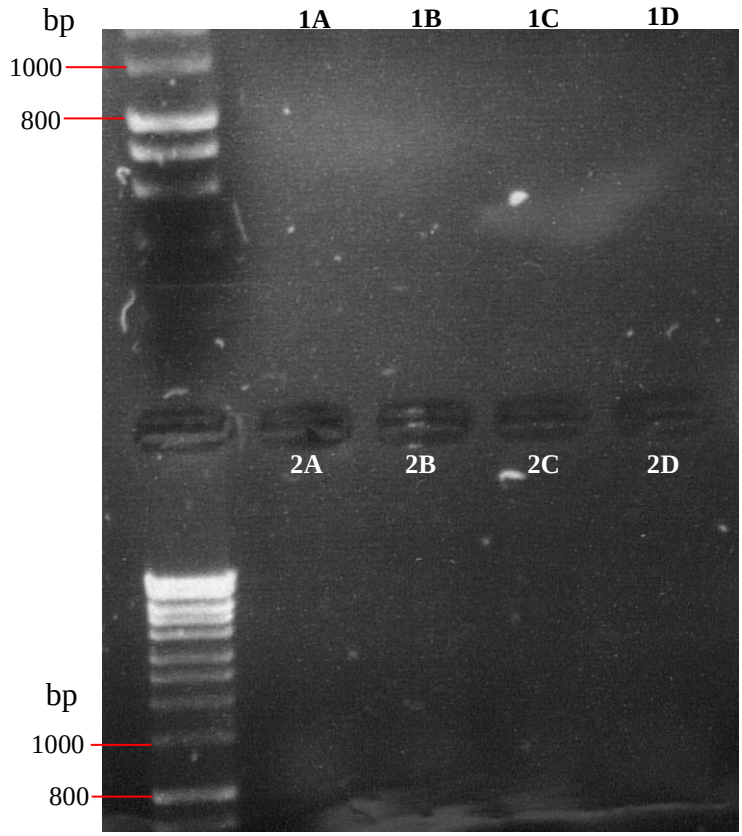
With the PCR continuing to give a low yield I checked the primer concentrations on the nanodrop, and the results are shown in table 6. This showed that the primers were not of the expected concentration following their dilution from the stock solution (10 µl of stock primer + 90 µl of nuclease free water) and a new diluted solution was made (*hrpA* 2 & *murE* 2), the new dilutions were used for all remaining reactions. In this PCR (figure 20) we also used a different DNA polymerase (Bioline MyFi DNA polymerase) that is designed to have a higher target affinity, an improved function in the presence of PCR inhibitors and a higher amplification sensitivity that is ideal for low copy number targets. The conditions for the PCR were:

- 95°C 5 mins (95°C 30s, 55°C 30s, 72°C 30 sec) x 30 and a final extension of 72°C 7 mins

- MyFi DNA polymerase 25 µl reaction mix:
    - 5 µl buffer
    - 1 µl forward primer
    - 1 µl reverse primer
    - 1 µl polymerase
    - 1 µl DNA sample
    - 16 µl nuclease free water

Table 6: *H. defensa murE* & *hrpA* diluted primer concentrations

Primer	Conc (ng/µl)	260/280	260/230
hrpA F	44.1	1.87	1.64
hrpA R	82.6	1.42	2.12
murE F	485.3	1.71	2.23
murE R	224.9	1.71	2.47



Lane No.	Polymerase	DNA sample ID	Primer ID
1A	MyFi	N116-1	*hrpA* 2
1B	MyFi	N116-2	*hrpA* 2
1C	MyFi	N116-1	*murE* 2
1D	MyFi	N116-2	*murE* 2
2A	MyTaq	N116-1	*hrpA* 2
2B	MyTaq	N116-2	*hrpA* 2
2C	MyTaq	N116-1	*murE* 2
2D	MyTaq	N116-2	*murE* 2

Figure 20: PCR 7
Image of the gel following PCR 7. The top lanes contain both DNA samples and primers with the MyFi DNA polymerase and the bottom lanes contain both DNA samples and primers with the MyTaq DNA polymerase. Neither DNA polymerase in this PCR managed to amplify the target sequence with the new dilution of primers.

11.3.6.1.9 PCR 8: MyFi & MyTaq DNA polymerase troubleshooting

MyFi troubleshooting

The MyFi polymerase recommends between 0.2-0.6 µM of each primer and 100 ng of DNA per 25 µl reaction so I altered the amount of the primers and DNA in each reaction as outlined in table 7, to test if changes in either concentration improved the outcome of the PCR. The DNA sample that I was using in this reaction (N116-1) had a concentration of 72.2 ng/µl so I would require a minimum of 1.38 µl of N116-1 (100/72.2) to meet the minimum requirement of template DNA for this polymerase if this sample was made up of just symbiont DNA.

Table 7: Components of PCR 8 reactions with the MiFi DNA polymerase

Lane No. (Fig 21)	1A	1B	1C	1D	1E	1F
Buffer vol (µl)	5	5	5	5	5	5
Template DNA vol (µl)	1.5	2	2.5	2	2	2
Forward primer vol (µl)	1	1	1	0.5	1	1.5
Reverse primer vol (µl)	1	1	1	0.5	1	1.5
Polymerase vol (µl)	1	1	1	1	1	1
Nuclease free water vol (µl)	15.5	15	14.5	16	15	14
Total reaction volume (µl)	25	25	25	25	25	25

MyTaq troubleshooting

The MyTaq also recommends that you use between 0.2-0.6 µM of both forward and reverse primers and 100 ng of DNA per 25 µl reaction. Table 8 outlines the different variations in the reaction mix in each PCR with the MyTaq ready mix.

Table 8: Components of PCR 8 reactions with MyTaq DNA polymerase

Lane No. (Fig 21)	2A	2B	2C	2D	2E	2F
PCR Red mix (µl)	12.5	12.5	12.5	12.5	12.5	12.5
Template DNA vol (µl)	1.5	2	2.5	2	2	2
Forward primer vol (µl)	1	1	1	0.5	1	1.5
Reverse primer vol (µl)	1	1	1	0.5	1	1.5
Nuclease free water vol (µl)	9	8.5	8	9.5	8.5	7.5
Total reaction volume (µl)	25	25	25	25	25	25

Both the MyFi and MyTaq PCR reactions used only the *H. defensa* specific *hrpA* primers and the N116-1 DNA sample, for simplicity (figure 21). As the Tm° of the primers are 61.8°C (forward) and 62.3°C (reverse) respectively we set the annealing temperature at 60°C.

The PCR conditions were:

- 95°C 5 mins (95°C 30s, 60°C 30s, 72°C 30 sec) x 40 and a final extension of 72°C 7 mins

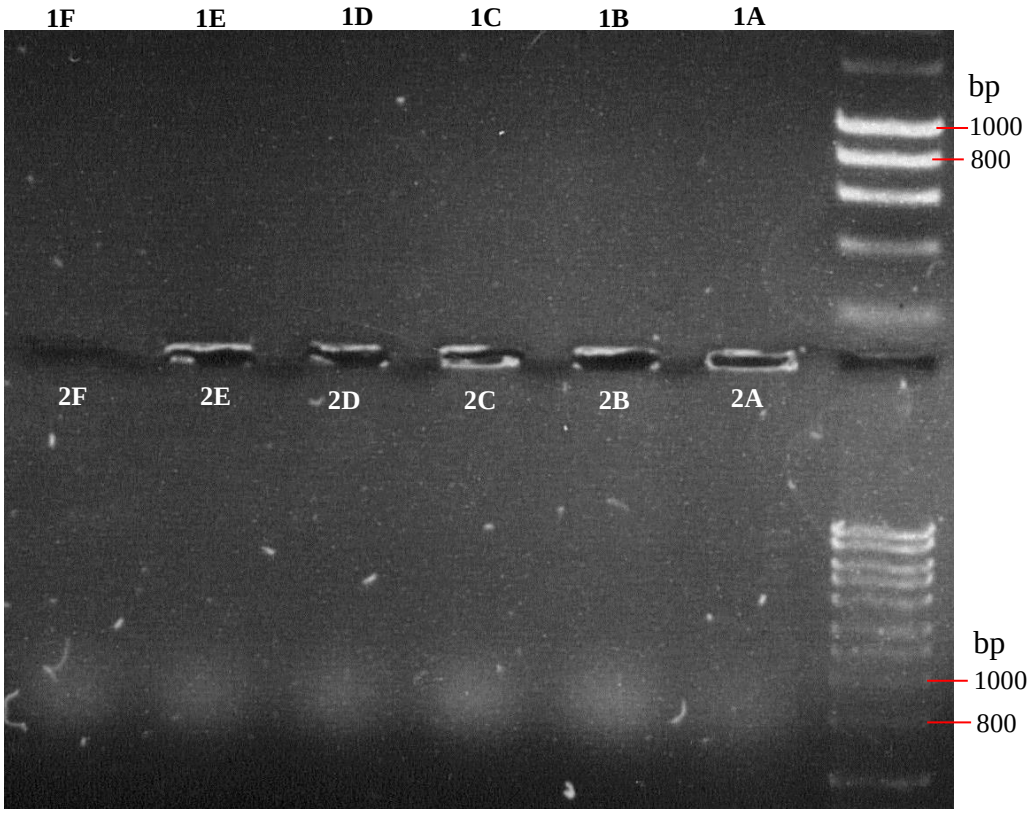


Figure 21: PCR 8

Image of the gel following PCR 8. The top rows are the reactions that used the MyFi and the bottom row are those that used the MyTaq. The exact composition of each reaction can be seen in tables 6 & 7. From the gel we can see that the MyFi reactions failed to amplify any of the *hrpA* gene and the MyTaq ready mix was able to amplify something. However, this does not constitute a positive result as the band is too weak.

11.3.6.1.10 PCR 9: *H. defensa* 16s specific primers

As I had continued to have problems with the *murE* and *hrpA* primers we then decided to try and use the *H. defensa* specific 16s primers, shown below in table 9, from the Henry et al paper (2013). The primers were diluted into a 1:10 solution before use and as the manufacturer's reported Tm° of the primers was not close I used a gradient PCR to ascertain their optimal annealing temperature. The increments of annealing temperature are set by the thermocycler (Biorad C1000) and are set out in table 10. The reaction conditions were:

- 95°C 3 mins (95°C 30s, *°C 30s, 72°C 30 sec) x 30 and a final extension of 72°C 7 mins
- *see table 10 for annealing temperatures.

Table 9: *H. defensa* specific 16s primer information

Gene	Primer names	Stock Conc (nmol)	Tm°	Sequence (5′-3′)
16s	16s- 10F	31.7	62.9	AGTTTGATCATGGCTCAGATTG
	16s- TO419R	31.0	54.7	AAATGGTATTSGCATTTATCG

*Primers were diluted from the stock concentration above to a 1:10 working mix that was used in the PCR reactions

Table 10: PCR 9 annealing temperatures by lane in gel
See figure 22

Lane No.	1A	1B	1C	1D	2A	2B	2C	2D
Annealing temperature (°C)	50	50.7	52	33.9	56.3	58.3	59.4	60

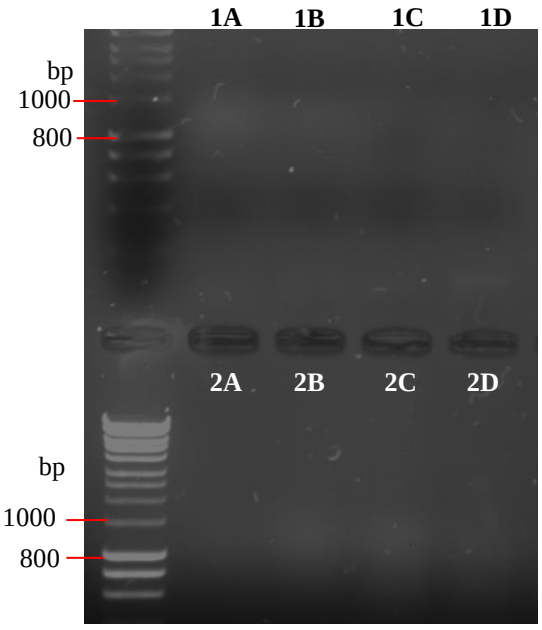


Figure 22: PCR 9 temperature gradient
The annealing temperature of each lane is shown in table 9 and all other conditions were the same. No bands were present in the gel and the PCR had failed to amplify anything.

The PCR gradient failed to produce a band at any of the temperatures (figure 22) and it was at this point I began to question if the problem was with the DNA samples and extracted DNA from the aphids again.

11.3.6.1.11 DNA extraction 2

The second extraction was taken from a sample frozen after the experiment, S31 D1, this sire × dam group was selected as the lab cultures were very low at this point and this group only produced 1 mummy after stage 1 of the experiment. As it had

produced 1 mummy, we could be sure that the wasp was functional, and the low number of mummies were likely to be the result of immunity. We had also managed to freeze a large number of aphids in this group. As previously mentioned, the defensive symbiont *H. defensa* is known to rapidly spread through lab populations of aphid after exposure to parasitoids I hoped that this would mean that there would be a greater number of this bacterium in this aphid population thus increasing the amount of the symbiont DNA extracted.

This extraction used the same method as the previous attempt but with a larger number of aphids (30), a sample of aphids was kept frozen for later molecular work. In this extraction, I also increased the lysis stage (step 3 in the insect supplementary protocol) from three to six hours. This DNA sample was labelled N116 3 and after the extraction, we checked the concentration of the DNA on the nanodrop (see table 11 for results). The data shows that this extraction had increased the concentration and purity of the DNA.

Table 11: DNA extraction 2 nanodrop data

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
N116 3	467.3	2.09	2.02

11.3.6.1.12 PCR 10: Successful amplification with all primers

After completing the extraction of N116 3 and getting an improved yield of DNA I tested all the primers again with this new sample. All reactions used the MyTaq RedMix, 1A and 1B were run on the Applied Biosystems 2720 thermocycler and the gradient PCR (lanes 1C – 2E) used a Bio-Rad C1000 thermocycler, with the increments of the temperature gradient being decided by the thermocycler program. For clarification, the 'Universal 16s primers' are 27F and 1492R (Weisburg et al., 1991) and the *H. defensa* specific 16s are 16s- 10F and 16s- TO419R (Henry et al., 2013). The conditions for the reactions were:

- 95°C 5 mins (95°C 30s, *°C 30s, 72°C 30 sec) x 30 and a final extension of 72°C 7 mins
- *see table 12 for annealing temperatures and primers used in each lane.

And the 25 µl reaction was made using:

- 12.5 µl PCR master mix
- 9.5 µl nuclease free water

- 1 µl forward primer
- 1 µl reverse primer
- 1 µl DNA sample

The gel showed a positive result with all primers (figure 23) including those specific to the defensive symbiont *H. defensa*, confirming its presence in the N116 pea aphid. The gradient PCR showed that the optimal annealing temperature of the *hrpA* primers was around 58.3°C.

Table 12: PCR 10
The table shows the primers and Annealing temperature used for each lane in the gel shown in figure 23

Lane No.	Primers ID	Annealing temperature
1A	Univeral 16s	60
1B	*H. defensa* specific 16s	60
1C	*hrpA*	60
1D	*hrpA*	59.4
1E	*hrpA*	58.3
2A	*hrpA*	56.3
2B	*hrpA*	53.9
2C	*hrpA*	52
2D	*hrpA*	50.7
2E	*hrpA*	50

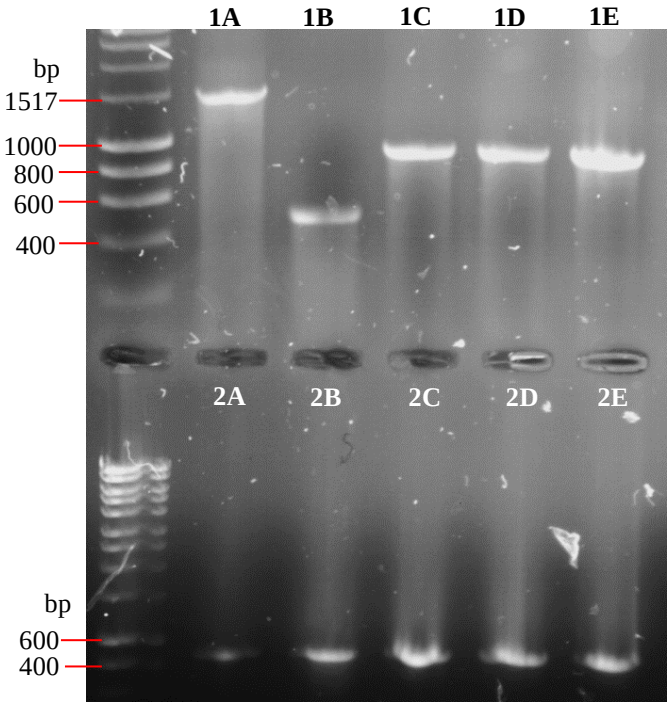


Figure 23: PCR 10
The gel following this PCR showed a positive result with all primers (16s specific, 16s universal, *hrpA*). This confirms the presence of *H. defensa* in the N116 aphid population. Lane 1E showed that the optimal annealing temperature of the *hrpA* primers to be around 58.3°C.

11.3.6.1.13 DNA extraction 3

With the conformation of the defensive symbiont in the N116 pea aphid, the next step was to test for the presence of *H. defensa* in the Quad pea aphid line. DNA was extracted from a quad aphid line used in the experiment (S6 D3) using the same protocol as in extraction 2 and the nanodrop results are shown in table 13.

Table 13: DNA extraction 3 nanodrop data

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
Quad 1	1355.5	2.14	2.19

11.3.6.1.14 PCR 11: Quad aphid diagnostic PCR

The Quad 1 DNA sample was then used with the same universal16s primers and the *H. defensa* specific primers used in PCR 10 to test for the presence of the defensive symbiont in this pea aphid line. PCR 11 was conducted with the same protocol as in PCR 10 and the result can be seen in figure 24. It shows that there was amplification of the universal 16s region in lane 1A but no amplification of the 16s region specific to the defensive symbiont, indicating that it is not present in this aphid line.

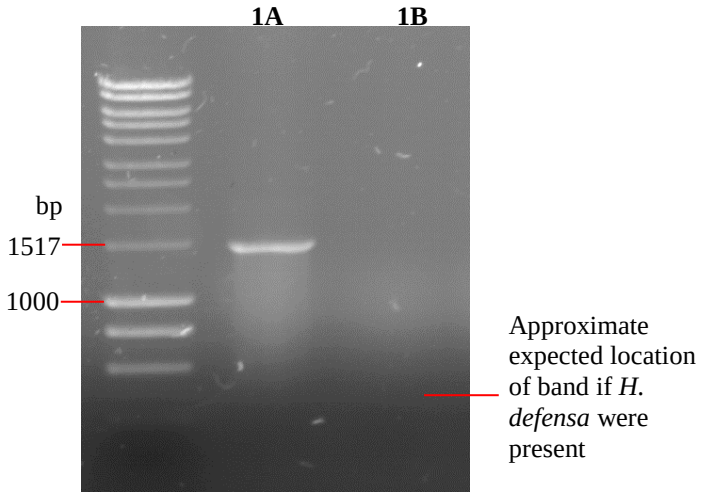


Figure 24: PCR 11
The gel showed a clear band in 1A (universal 16s) and no band in 1B (*H. defensa* specific 16s), confirming that the defensive symbiont is not present in the Quad pea aphid line.

11.3.6.2 16s gene sequencing

In order to identify the other symbionts of the N116 and Quad aphid lines, I used a combination of general bacterial 16s rRNA gene primers, 27F and 1492R (Weisburg et al., 1991) to amplify the 16s region of all the aphids bacterial symbionts. I then used the amplicon in a cloning and transformation reaction, the details of which are in the proceeding sections, and extracted plasmids for the sequencing.

11.3.6.2.1 **DNA extraction 4 with surface sterilisation**

As the method that I had chosen for sequencing was not specific and would amplify the DNA of all bacteria, it was important to sterilise the surface of the aphids before extracting the DNA as this ensures that you only extract DNA from the bacteria found inside the aphids. The aphids used in this extraction were members of the S31 D1 (N116) and S6 D3 (Quad) groups used in the previous extraction. The method used to sterilise the aphid's surface is outlined below (Leroy et al., 2011):

1. The ethanol and NaCl solutions were made using autoclaved MiliQ water and then filtered using a 0.45 µm millex syringe filter
2. The aphid's whole bodies are washed by submerging them in a sterile 70% ethanol solution for 3mins.
3. The ethanol is poured away and then the aphids are washed in a 9g l^{-1} NaCl solution.
4. A final wash with autoclaved MilliQ water removes any contaminants that could interfere with the sequencing.

Once the aphids had been surface sterilised the DNA was extracted using the same method as DNA extraction 2 and they were labelled 1) N116 4 and 2) Quad 2 and their concentrations were measured on the nanodrop (data in table 14).

Table 14: DNA extraction 4 Nanodrop data

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
Quad 2	275.9	2.06	1.79
N116 4	177.1	2.02	1.55

11.3.6.2.2 PCR 12: Preparation of 16s DNA for transformation 1

In this PCR I amplified the relevant regions of the 16s rDNA that were needed for the sequencing and included various others as a control (outlined in table 15). The gel shows a repeat of the N116 3 and Quad 1 samples with both the *H. defensa* specific and universal 16s primers and the surfaced sterilised N116 4 and Quad 2 samples with the same primers. This meant that we could be sure that the PCR had been successful and if the new samples were negative it would be indicative of a problem with the DNA samples, the result of PCR 12 can be seen in figure 25. The conditions for the 25 µl PCR were:

- 95°C 5 mins (95°C 30s, 60°C 30s, 72°C 30 sec) x 30 and a final extension of 72°C 7 mins

And the 25 µl reaction was made using:

- 12.5 µl PCR master mix
- 9 µl nuclease free water
- 1 µl forward primer
- 1 µl reverse primer
- 1.5 µl DNA sample

The amount of template DNA was increased to improve the yield of the amplicon. Once the gel had finished bands 2A and 2C were cut out of the gel with a sterile scalpel and extracted using the Qiagen QIAquick gel extraction kit and quick-start protocol and then stored at -20°C. The nanodrop data is shown in table 16. The Nanodrop data showed that we had a very low quantity of nucleic acid, as a result,

the transformation was unlikely to be successful and I decided to repeat the amplification and try and purify the bands again.

Table 15: PCR 12
The table shows the contents of each lane in PCR 12 (figure 25)

Lane No.	Primers	DNA sample ID
1A	Universal 16s	N116 3
1B	Specific 16s	N116 3
1C	Universal 16s	Quad 1
1D	Specific 16s	Quad 1
2A	Universal 16s	N116 4
2B	Specific 16s	N116 4
2C	Universal 16s	Quad 2
2D	Specific 16s	Quad 2

Table 16: Nanodrop data of gel extracted 16s gene from PCR 12

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
Quad 2 universal 16s A	5.9	2.21	0.01
N116 4 universal 16s A	16.9	1.90	0.04

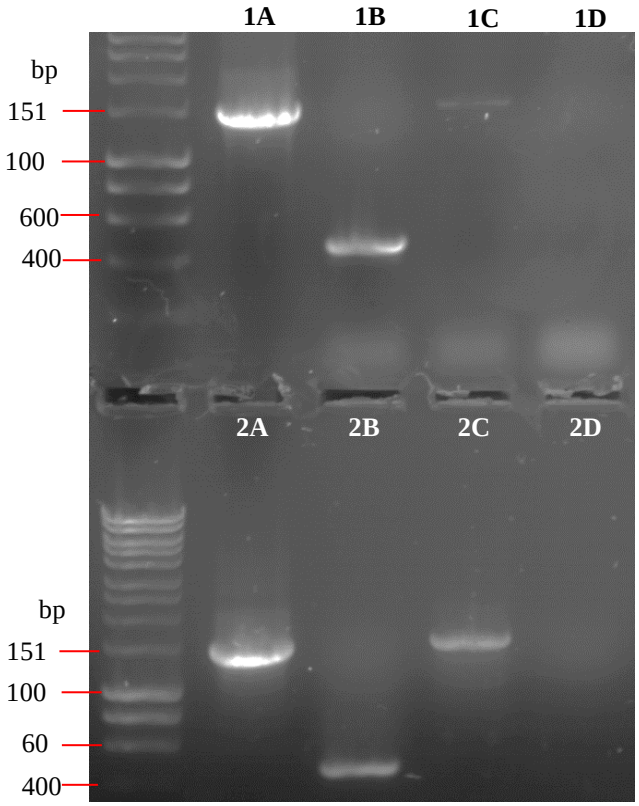


Figure 25: PCR 12
The PCR worked as anticipated and amplified in all regions where we expected to find bands. The full breakdown of the components of each lane is given in table 15.

11.3.6.2.3 PCR 13: Preparation of 16s DNA for transformation 2

To further increase the quantity of the amplicon that was available to extract from the gel, I increased the loading volume of the gel from 25µl to 50µl by doubling all the reaction mix components used in the previous PCR and increased the number of cycles from 30 to the thermocycler's maximum, 40. The middle lane (figure 26) was left empty in order to make the extraction of the bands easier. The reaction components and conditions were:

- 95°C 5 mins (95°C 30s, 60°C 30s, 72°C 30 sec) x 40 and a final extension of 72°C 7 mins
- 25 µl PCR master mix
- 18 µl nuclease free water
- 2 µl 27F forward primer
- 2 µl 1492R reverse primer
- 3 µl DNA sample

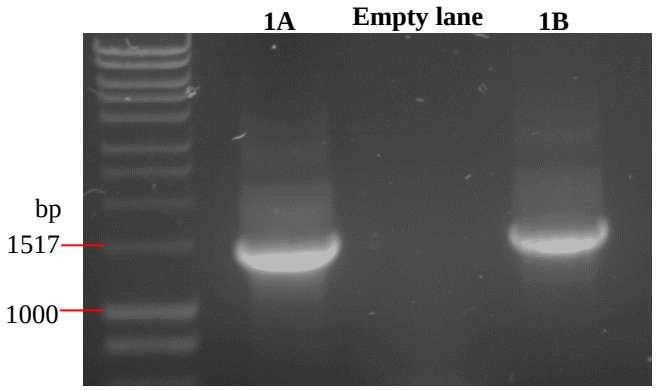


Figure 26: PCR 13
Lane 1A is the N116 4 DNA sample and 1B is the Quad 2 DNA sample, both with the universal 16s primers before their extraction from the gel.

Once the gel electrophoresis had finished the bands were again cut from the gel using a sterile scalpel and extracted using the Qiagen QIAquick gel extraction kit. The sample was then quantified on the nanodrop and data can be found in table 17. Again, the yield of the 16s gene that I had extracted from the gel was very low, but I decided to continue with the transformation reactions using the 'Quad 2 universal 16s B' and 'N116 4 universal 16s A' samples as these had the highest nucleic acid concentration. However, despite the 260/280 ratios of my samples being high and the 260/230 ratios being much lower than expected, this could have been as a result of the very low concentration of nucleic acid in the samples. The nanodrop accuracy is significantly reduced at the lower end of the detection range (approx. 10 ng/µl).

Table 17: Nanodrop data of gel extracted 16s gene from PCR 13

Sample ID	Nucleic acid (ng/µl)	260/280	260/230
Quad 2 universal 16s B	9.8	3.21	0.01
N116 4 universal 16s B	6.9	2.96	0.01

11.3.6.2.4 Ligation and transformation 1

The ligation of my samples into a plasmid was conducted using the Promega 'pGEM®-T Easy Vector System 1' as per the manufacture's protocol and set up in the following reaction and left overnight at 4°C. As our insert was at a very low concentration, we had to add the maximum quantity of PCR product (3 µl) and reduce the dilution with nuclease free water to 0µl.

- 5 µl Buffer
- 1 µl Vector
- 3 µl PCR product (Quad 2 universal 16s B & N116 4 universal 16s A)

- 1 µl T4 DNA ligase
- 0 µl Nuclease free water

The transformation reaction used XL1-Blue competent cells (Agilent Technologies) and the manufactures 'transformation protocol' with some slight alterations. I added 2 µl of the ligated plasmid sample to the aliquot of cells (Step 5). I also used LB media instead of the suggested SOC media (step 9) and the LB-ampicillin plates were made by adding 15g of agar and 25g of LB medium to 1L of MiliQ water and mixing well. The LB agar was then autoclaved and left to cool to below 55°C. When the mixture reached the correct temperature, it was taken to a laminar flow cabinet and the filter-sterilized ampicillin was added (1 ml of a 100mg/ml solution). The agar is then mixed again and poured into petri dishes and left to set in the laminar flow, under flame. For colour screening, 100µl of 100 mM IPTG and 20µl of 50 mg/ml X-gal was petted onto the agar and spread evenly (step 10). The plates were left for 48 hours for the colonies to develop as they were too small after the suggested 17 hours.

After 48 hours of incubation at 37°C the colonies where incubated at 4°C for 2 hours to enhance the colours. The growth of any bacteria that did not take up the plasmid would be inhibited by the antibiotic and if a colony formed and contained the plasmid but not the 16s gene I had tried to insert, then the colonies grow on the plates and appear blue in colour. Moreover, if the colonies contain the plasmid with the 16s gene insert, they appear white, making the colonies easy to sample. The colonies were sampled by touching them with a pipette tip and the tip was dropped into a falcon tube that contained 3ml of sterile LB-ampicillin media (same concentration of antibiotic as the plates). The sampled colonies were incubated at 37°C, with shaking at 225-250 rpm, overnight. The next day the plasmids were extracted from the sampled colonies using the Qiagen, QIAprep® spin miniprep kit and quick start protocol and eluted with nuclease free water. However, on the first attempt at sampling the colonies very few of them grew successfully in the liquid LB-ampicillin media (50% of those sampled).

I suspected that the low success rate of the colonies in the liquid media was likely due to either the colour screening, as the colonies were very hard to distinguish or because of the antibiotic that I had used in the LB-ampicillin agar. It had been made previously and stored at -4°C for a few months before I had used it whereas the antibiotic that was used in the liquid media was a new dilution as there was no more

of the premade dilution left. However, the samples that I had extracted were checked on the nanodrop and were of sufficient quality for sequencing. The ligation, transformation and plasmid extraction processes were repeated, and more colonies were extracted but there was a limited number of colonies that clearly passed the colour screening and that could be extracted. The process was repeated 3 more times and bacterial colonies were sampled until I had at least 35 samples from both the quad and N116 aphid lines.

11.3.6.2.5 **Preparation for sequencing**

Before the plasmids were sent for sequencing, 15 samples of each aphid line were digested with the restriction enzyme EcoR1. This enzyme removes the insert sequence from the plasmid we had used and enabled us to visually confirm its presence before sequencing. After digestion, when the samples were run on a 1% agarose gel, they showed 2 clear bands, one for the plasmid and a band of a similar size to the insert. Unfortunately, the printer on the transilluminator was faulty and I was unable to get a photograph of the result. To prepare the samples for sequencing they were checked on the nanodrop. GATC Biotech AG, London UK, (now Eurofins GATC) recommended a DNA concentration of between 30 – 80 ng/µl for their plasmid 'Supreme run' Sanger sequencing. Those samples that had a concentration higher than recommended were then diluted, with nuclease-free water, to a final concentration of 60ng/µl. A total of 70 samples (35 quad & 35 N116) were sent for sequencing using GATC Biotech's T7 sequencing primers.

11.3.6.2.6 **Analysis of sequencing data**

Once we had received the sequence data both the vector sequences and the parts of the sequences that contained lots of bases that were below the confidence threshold (shown in the sequence as lower-case letters or 'n') were removed (an example of this is shown below). The quality value (Q) of each base is a number (from 4 to approximately 60) is a statistical assessment of the accuracy of each base in the sequence, with higher Q values representing higher quality. It is calculated by taking the $\log_{10}$ of the error probability × 10, a Q score of 10= a 1 in 10 probability that the base called is incorrectly (90% accuracy), a Q 20= a 1 in 100 chance that the base called is incorrectly (99% accuracy) and Q20 is the accepted threshold for Sanger sequencing (Ewing and Green, 1998; Ewing et al., 1998). The sequences

were then analysed using the NCBI ‘standard nucleotide BLAST’ (megablast) and the Nucleotide collection (nr/nt). The most closely related bacteria were selected based on the blast output and where they fall on the resulting distance tree of the results. The full output of the blast analysis can be found in section 14.1 p103 (Appendix 1).

11.3.6.2.7 **Naming and trimming of sequences**

The raw sequences that we received from GATC were trimmed as followed. For reference, the samples were named in the following way so that they could be traced.

e.g. ID: N100_4-T7

- N= N116 clone
- 100= the 100th bacterial colony from that particular agar plate I sampled
- 4= the plate the bacterial colony was produced with the 4th transformation reaction (in other words the repeat from which the sample is taken)
- T7= is the set of sequencing primers that I used (same for all samples).

Raw sequence from GATC

Yellow highlighted deleted vector sequences

Green highlighted sequences deleted as they contain many bases that did not pass the confidence threshold

Sample ID: N2_2-T7

gCtccGGCCGccAtGGCGGCCGCGGgAaTTCGATTAGAGTTTGATCCTGGC
TCAGATTGAACACTGGTGGCAGGCCTAACACATGCAAGTCGAGCGGCA
TCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCGAGCGGCGGACGGG
TGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGGATAACTGCTGGAA
ACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAAGTGGGGGACCTT
CGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTAGCTGGTAGGTA
AGGTAAGGGCTTACCTAGGCGACGATCTCTAGCGGGTCTGAGAGGATA
GCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCCATGCC
ACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAG
GAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCG
AGCGTTGATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAGTT
AAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAAC
TGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGG
TGAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC
TGGagAAAGACTGACGCTGAGGTGCGAAAGCGTGGGgAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCTGTAAACGATGTcgATTTGgaGGTTGC
GGTCTTGAACTGTGgCGTCCGGAGCTAACGCGttAAaTCGACCGCctGGgg
gAGTACGGCccgcaaGgTTAAAACTCAAATgaaaTtGACGGGGGgccnncACA

AGcggTGGaagcATGTGGgTtnatttcgatgnAacGnnnaanAaCC

The final sequence used in the BLAST analysis

>ID: N2_2-T7

AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAGGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTGATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GagAAAGACTGACGCTGAGGTGCGAAAGCGTGGGgAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCTGTAAACGATGTcgATTTGgaGGTTGCGGTC
TTGAACTGTGgCGTCCGGAGCTAACGCG

11.3.7 Statistical analysis

Because of the added complication of the immunity in the N116 aphid we had to break down our statistical analysis into many levels, as shown in this section. All the statistical analysis and data visualisation were done using R (R Core team, 2017) via Rstudio (RStudio Team, 2015). As the number of sibling groups that we were able to split, between presence (+IGP) and absence (−IGP) of Intraguild predation (IGP), were so few (only 10 sibling groups) the analysis was broken down into the following levels. We first analysed the data from all the microcosms that were exposed to the wasp genotypes but not the IGP, then all the data from the aphids exposed to both the wasp genotypes and the IGP and then the data of the 10 sibling groups that were split between the absence and presence of IGP.

11.3.7.1 Analysis 1: interspecific indirect genetic effect and intraguild predation, the effect of genetic variation in the parasitoid on the N116 genotype, in the presence and absence of IGP.

11.3.7.1.1 **Trait 1: Parasitoid virulence in the absence of IGP**

The response variable, parasitoid virulence, is explained by the proportions of aphids that were mummified after 11 days of exposure to the female parasitoid in relation to the entire population of aphids per microcosm. The parasitoid virulence, in each of the aphid lineages, is a continuous numerical variable and was comparatively quantified using the following calculation: (no. mummies × 100 / total no. aphid (healthy + mummified)) and visualised using R. A generalised linear model was used with quasipoisson family due to non-normality and over-dispersion, R package (multcomp) (Hothorn et al., 2008). The explanatory variables were as follows:

1) Aphid immunity (yes or no). The yes category contained all the instances where the aphids showed no sign of mummification after 11 days of exposure to the parasitoid in each individual microcosm.
2) Sire effect (sire identity).
3) Dam effect (dam identity).
4) Parasitoid genotype (daughters' identity [sibs and $_{1/2}$ sibs] that are the product of the nested $_{1/2}$ sib quantitative genetic design).

Aphid immunity, in these analyses, is a categorical variable (no= 0; yes= 1) that refers to the occurrence of mummification in the aphids and should be thought of as 'total immunity' within the microcosm. The presence of mummies indicates that the female wasp was successful in producing offspring, leading to the death of an aphid host. The absence of any mummification reflects a major fitness failure of the parasitoid and indicates high immunity of the aphid host, where the aphid was able to avert the complete parasitoidisation. The inclusion of aphid immunity as an explanatory variable provides an insight into why there was an absence of mummies in certain lines despite us observing successful oviposition.

11.3.7.1.2 **Trait 2: Mummification position in the absence of IGP**

The position of the mummies represents a measure of aphid behaviour in our experiment. We analysed the position data in a hierarchal multinomial regression linear model with multinom family, R packages 'nnet' (Venables and Ripley, 2002)

and ‘car’(Fox and Weisberg, 2011). The response variable was mummy positions: off-plant and on-plant (on-plant is broken down into one of three plant strata: top, middle and bottom) after 11 days of exposure to the daughter parasitoid per microcosm. The on-plant versus off-plant distribution of mummies is an indicator of the altruistic adaptive suicide trait in aphids, where they expose themselves to their natural enemies.
The explanatory variables were as follows:

1) Sire effect (sire identity).
2) Dam effect (dam identity).
3) Parasitoid genotype (the daughter identity, consisting of sibs and $_{1/2}$ sibs that were produced by the nested half sibling quantitative genetic design).

11.3.7.1.3 Part 2: N116 aphid exposed to IIGE in the presence of IGP

In this analysis, we used the data from the microcosms that all contained the IGP. All the model explanatory and response variables, for both traits (parasitoid virulence and mummy position), is the same as section 11.3.7.1.1 & section 11.3.7.1.2.

11.3.7.1.4 Part 3: N116 aphid exposed to IIGE in comparison with IGP

In this analysis, we used the limited data from the small number of parasitoid sibling groups that we were able to split, with half of the daughters (genotypes) being exposed to IGP and the other half that were not exposed to IGP (i.e. IIGE only). This enabled us to understand the influence that IGP has on the outcome of the IIGE. The explanatory and response variables for both parasitoid virulence and mummy position traits are the same as section 11.3.7.1.1 & section 11.3.7.1.2 but with the added explanatory variable IGP (yes or no).

11.3.7.2 Analysis 2: genetic variation in both the aphid (Quad and N116) and the parasitoid under the influence of an indirect ecological effect (defensive symbiont)

In this section we had the same two response variables: the first was wasp virulence (as in section 11.3.7.1.1). The second response variable was the mummies’ position (as in section 11.3.7.1.2). For all the sections described below, the wasp virulence

was tested using generalised linear models with a quasipoisson family due to non-normality and over-dispersion, R package ‘multcomp’ (Hothorn et al., 2008). Moreover, for mummy position we used a hierarchal multinomial regression linear models with a multinom family, R packages ‘nnet’ (Venables and Ripley, 2002) and ‘car’ (Fox and Weisberg, 2011).

11.3.7.2.1 **Trait 1: Parasitoid virulence**

In this analysis, we used the data from both clones of pea aphid: The Quad clone (vulnerable to parasitoid) and the N116 clone (highly immune) from the microcosms that did not contain an IGP. The explanatory variables were as follows, note that we now have the added variable of aphid genotype (N116 or Quad):

1) Aphid immunity (yes or no). The yes category contained all the instances where the aphids showed no sign of mummification after 11 days of exposure to the parasitoid in each individual microcosm.
2) Aphid genotype (N116 or Quad).
3) Sire effect (identity of the sire).
4) Dam effect (identity of the dam)
5) Parasitoid genotype (daughters [sibs and $_{1/2}$ sibs] that are the product of the nested $_{1/2}$ sib quantitative genetic design).

11.3.7.2.2 **Trait 2: Mummification position**

This analysis also made use of the data we collected from each of the aphid genotypes in the absence of IGP and the explanatory variables were as follows:

1) Aphid genotype (N116 or Quad).
2) Sire effect (sire identity).
3) Dam effect (dam identity).
4) Parasitoid genotype (the daughter identity, consisting of sibs and $_{1/2}$ sibs that were produced by the nested half sibling quantitative genetic design).

11.4 Results

11.4.1 Phenotypic data

As a result of the difficulty in establishing the quantitative genetic design, discussed in the methods, where we had low numbers of daughters and, as a result, were not always able to split the family groups between the exposure to IGP (+IGP) and the absence of IGP (−IGP). To counter this, we first analysed the data collected from the microcosms that were not exposed to IGP and, then separately analysed the data from those that were exposed to IGP (first two analyses). We then analysed the small number of daughter groups (just 10) where we were able to split family groups in half between the +IGP and −IGP. Finally, we compare the data from the N116 clone (−IGP) to the Quad clone (−IGP).

11.4.1.1 N116 exposed to IIGE without IGP

The only factor that was significant in its effect on wasp virulence was aphid immunity ($F_{(1,29)}$ = 17.19, P= 0.002), showing a negative influence and contributing to 49% of the explained variance. With regards to the mummification position, we found that the sire effect was the only significant factor effecting mummification position (X^2= 42.89, P= 0.003), contributing to 86% of the explained variance.

Table 18: Parasitoid virulence analysis 1- N116 exposed to IIGE −IGP
GLM summary of parasitoid virulence. Response variable: parasitoid virulence and the predictors: 1) Immunity (yes or no), 2) sire identity, 3) dam identity, 4) parasitoid genotype identity.

Response: Parasitoid Virulence			
Predictors:			
	F	**Df**	**P**
Immunity	17.1876	29	**0.001628**
Sire	0.3524	22	0.911745
Dam	1.5443	15	0.249259
Parasitoid Genotype	1.1573	11	0.380899

Table 19: Mummification position analysis 1- N116 exposed to IIGE −IGP
Hierarchical multinomial model (using likelihood ratio) summary. Response variable: mummy position and the predictors: 1) sire identity, 2) dam identity, 3) parasitoid genotype identity.

Response: Mummy Position			
Predictors:			
	LR Chisq	**Df**	**P**
Sire	42.887	21	**0.003249**
Dam	5.458	21	0.999730
Parasitoid Genotype	1.603	12	0.888814

11.4.1.2 N116 exposed to IIGE and IGP

Again, wasp virulence was significantly affected by aphid immunity ($F_{(1,41)}$ = 37.29, P<0.0001) but, here it was also affected by the identity of the dam (maternal effect) ($F_{(4,26)}$ = 4.68, P= 0.011) with each contributing 52% and 26% respectively to the explained variance. In this case, it was the individual parasitoid's genotype, rather than the sire effect, that was most significant in its effect on the position where the aphids mummified (X^2= 27.77, P= 0.023), contributing to 70% of the explained variance.

Table 20: Parasitoid virulence analysis 2- N116 exposed to IIGE & IGP
GLM summary of parasitoid virulence. Response variable: parasitoid virulence and the predictors: 1) Immunity (yes or no), 2) sire identity, 3) dam identity, 4) parasitoid genotype identity.

Response: Wasp Virulence			
Predictors:			
	F	**Df**	**P**
Immunity	37.2871	41	< **0.0001**
Sire	0.6408	30	0.77049
Dam	4.6765	26	**0.01084**
Parasitoid Genotype	0.8143	16	0.62016

Table 21: Mummification position analysis 2- N116 exposed to IIGE & IGP
Hierarchical multinomial model (using likelihood ratio) summary. Response variable: mummy position and the predictors: 1) sire identity, 2) dam identity, 3) parasitoid genotype identity.

Response: Mummy Position			
Predictors:			
	LR Chisq	**Df**	**P**
Sire	5.5908	18	0.99759
Dam	6.1310	12	0.90933
Parasitoid Genotype	27.7695	15	**0.02306**

11.4.1.3 Split N116 groups: IIGE −IGP contrasted with IIGE + IGP

The aphid immunity was again the main factor affecting the wasp virulence, contributing to 60% of the explained variance $F_{(1,17)}$ = 92.77, P<0.0001). There was also a sire effect ($F_{(4,13)}$ = 10.18, P=0.002) contributing 26% of the explained variance, and an effect of the individual parasitoid genotype ($F_{(2,9)}$ = 4.71, P= 0.039) showing 6% of the explained variance. The presence of IGP also had a significant effect on parasitoid virulence ($F_{(1,18)}$ = 8.27, P=0.018) contributing 5% to the explained variance. Moreover, Figure 27 shows that, excluding G1, G6 and G7, intraguild predation acted to reduce parasitoid virulence.

The explanatory variables had no significant effects on mummification positions. However, whilst the effect of IGP was not significant in predicting mummy position it was marginal (P= 0.09). From figure 28 we can see that the majority of mummies were found off-plant, this is likely the result of an increase of the adaptive suicide behaviour in these genotypes. However, further work would be required to confirm this.

Table 22: Parasitoid virulence analysis 3- N116 IIGE −IGP contrasted with IIGE + IGP
GLM summary of parasitoid virulence. Response variable: parasitoid virulence and the predictors: 1) IGP (yes or no) 2) Immunity (yes or no), 3) sire identity, 4) dam identity, 5) parasitoid genotype identity.

Response: Wasp Virulence			
Predictors:			
	F	**Df**	**P**
IGP	8.2664	18	**0.018323**
Immunity	92.7724	17	< **0.0001**
Sire	10.1809	13	**0.002139**
Dam	1.8423	11	0.213472
Parasitoid Genotype	4.7134	9	**0.039771**

Table 23: Mummification position analysis 3- N116 IIGE −IGP contrasted with IIGE + IGP
Hierarchical multinomial model (using likelihood ratio) summary. Response variable: mummy position and the predictors: 1) IGP (yes or no) 2) sire identity, 3) dam identity, 4) parasitoid genotype identity.

Response: Mummy Position			
Predictors:			
	LR Chisq	**Df**	**P**
IGP	6.3433	3	**0.09605**
Sire	12.2131	12	0.42872
Dam	4.4495	12	0.97389
Parasitoid Genotype	6.3341	6	0.38682

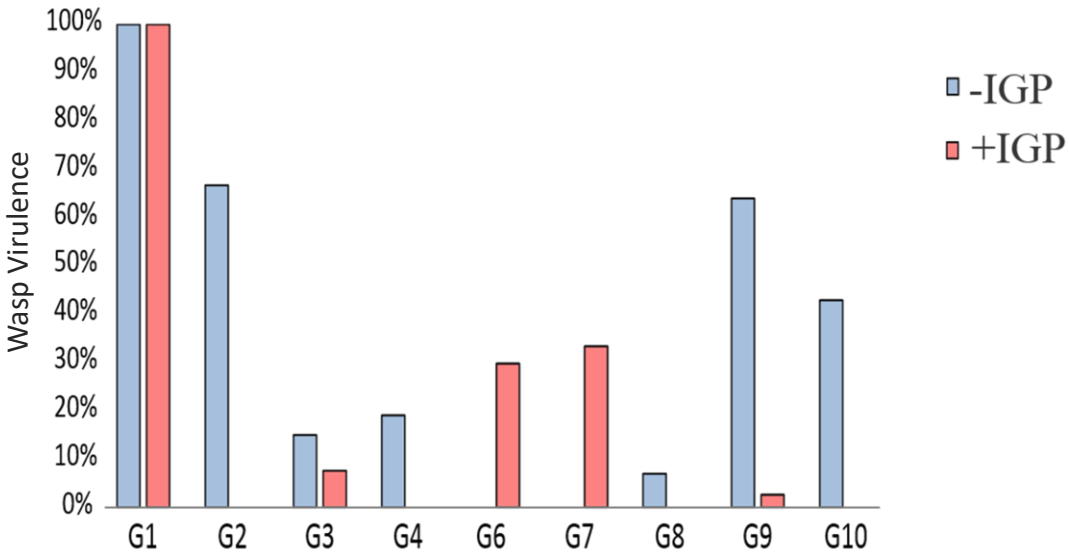


Figure 27: Comparison of wasp virulence +/- IGP
Wasp virulence across the 10 split family groups, generally the presence of and intraguild predator reduces wasp virulence. With G1, G6 and G7 representing outlying results.

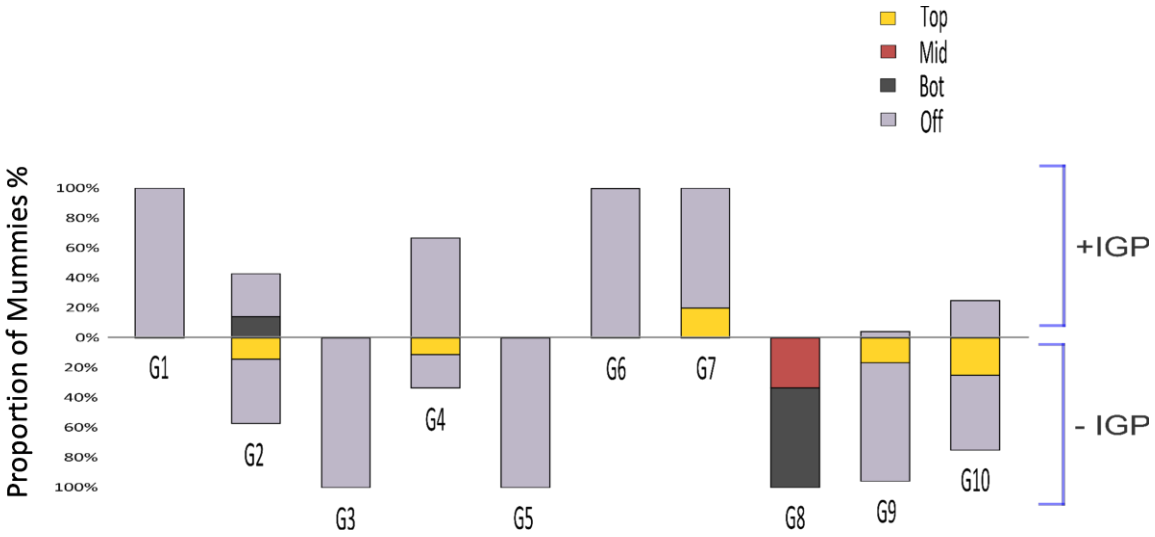


Figure 28: The proportions of N116 mummies and their positions
The genotypes shown here are the 10 dam groups where we had enough daughters to split them and expose half to intraguild predation (IGP) and the other half were not. The top row are the daughters that were exposed to IIGE (*A. ervi*) and IGP and the bottom row were exposed to the IIGE (*A. ervi*) alone. In both cases most of the aphid mummies were found off plant, this is likely to be the result of the aphid's adaptive suicide behaviour.

11.4.1.4 N116 aphid vs Quad aphid

The virulence of the wasp was again significantly affected by aphid immunity ($F_{(1,58)}$ = 58.24, P<0.0001) with a 75% contribution to the explained variance. Here, the aphid genotype also had a significant effect on the wasp virulence ($F_{(1,57)}$ = 12.47, P=0.001) with 16% contribution to the explained variance.

The position of the mummies on the plant was significantly affected by aphid genotype (X^2= 36.87, P<0.0001) and parasitoid genotype (X^2= 69.53, P<0.0001), With each contributing to 32% and 60% to the explained variance respectively.

Table 24: Parasitoid virulence analysis 4- N116 aphid vs Quad aphid
GLM summary of parasitoid virulence. Response variable: parasitoid virulence and the predictors: 1) immunity (yes or no), 2) aphid genotype (N116 or Quad), 3) sire identity, 4) dam identity, 5) parasitoid genotype identity.

Response: Wasp Virulence			
Predictors:			
	F	**Df**	**P**
Immunity	58.2393	58	< **0.0001**
Aphid Genotype	12.4646	57	**0.0008894**
Parasitoid Genotype	1.3062	52	0.2760927
Immunity: Aphid Genotype	0.0940	51	0.7603975

Table 25: Mummification position analysis 4- N116 aphid vs Quad aphid
Hierarchical multinomial model (using likelihood ratio) summary. Response variable: mummy position and the predictors: 1) aphid genotype (N116 or Quad) 2) sire identity, 3) dam identity, 4) parasitoid genotype identity.

Response: Mummy Position			
Predictors:			
	LR Chisq	**Df**	**P**
Aphid Genotype	36.869	3	< **0.0001**
Parasitoid Genotype	69.526	15	< **0.0001**
Aphid Genotype x Parasitoid Genotype	9.011	15	0.877

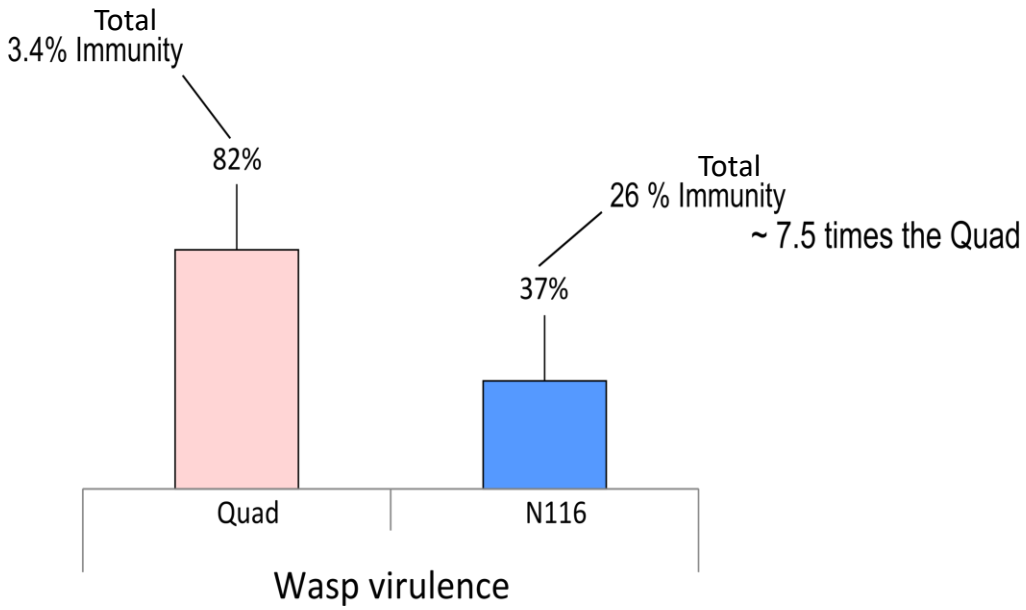


Figure 29: Comparison of wasp virulence with each aphid genotype
When you compare the levels of wasp virulence between the two aphid clones, you can see that the wasp virulence is reduced in the N116 pea aphid. The total immunity refers to the percentage of cases where no mummies were observed within the microcosm, and it 7.5 times higher in the N116 aphid than in the Quad. These two factors confirmed that there was a high level of immunity in the N116 pea aphid.

11.4.2 Molecular data

11.4.2.1 N116 aphid symbionts

A full summary of the N116 aphid 16s rRNA gene sequences and BLAST analysis results can be found in appendix 1 (section 14.1.1) and a summary is presented here. Of the 35 samples that were sequenced, 26 were successful and contained a long enough sequence to conduct a BLAST analysis. One sample matched with the aphid obligate symbiont *Buchnera aphidicaola*, that produces amino acids the aphid cannot produce and is essential for reproduction (Douglas, 1998; Oliver et al., 2003). Fifteen samples matched with the known defensive secondary symbiont *Hamiltonella defensa* that provides immunity to parasitoids (Oliver et al., 2005). We also found that nine samples were most closely related to *Fukatsuia symbiotica*, previously referred to as the X-type or PAXS symbiont, that when found in association with *H. defensa* provides high levels of resistance to *A. ervi* (Bilodeau et al., 2013; Manzano-Marín et al., 2017). Interestingly we also found that one sequence was most closely related to *Serratia symbiotica*, another known symbiont of aphids that provides resistance against parasitoids(Oliver et al., 2007; Oliver et al., 2005; Oliver et al., 2006; Oliver et al., 2003).

11.4.2.2 Quad aphid symbionts

For a full summary of the Quad aphid 16s rRNA gene sequences and BLAST analysis see appendix 1 (section 14.1.2) and a summary is presented here. In this aphid clone, I sent another 35 samples for sequencing and received 23 sequences of sufficient quality for BLAST analysis. Three samples positively matched with the obligate symbiont *Buchnera aphidicola* and 20 samples matched with the secondary symbiont *Serratia symbiotica*.

11.5 Discussion

Our findings have shown that attack by the parasitoid wasp can lead to changes in aphid behaviour. Khudr et al (2013) found that genetic variation in the parasitoid was associated with variations in host behaviour (the position of mummies) and thus represented an interspecific indirect genetic effect (IIGE). However, this experiment was limited to just one pea aphid genotype, the JF01/29 genotype, and the outcome of this indirect effect may differ between aphid clones. In this experiment, and in contrast to Khudr et al (2013), we found that in the N116 aphid clone, and in the absence of intraguild predation (IGP), there was an effect of the sire identity, rather than the parasitoid genotype, on aphid behaviour. Moreover, when the N116 aphids were exposed to IGP, there was an effect of the individual parasitoid genotype on the behaviour of the aphid.

This suggests that the interaction between the aphid and parasitoid is highly context-dependent and more complex than previously thought. This is further supported by our comparison of the N116 and Quad aphid genotypes where we found a significant effect of the parasitoid genotype on mummy location. We also found an effect of the aphid genotype on the location of the mummies, indicating that the response, of specific lineages of pea aphid, to the IIGE is also dependent on the specific genotypes involved. However, it is still not clear if the change in aphid mummy position (wasp puparia) is to the benefit of the host or parasitoid and is also likely to be context-dependent (Brodeur and McNeil, 1989; Carius et al., 2001; Khudr et al., 2013; McAllister et al., 1990; Trail, 1980).

Furthermore, an indirect ecological effect (IEE) is defined as an interaction between species that is altered or mediated by the presence of a third species (Rowntree et al., 2011; Strauss, 1991; Wootton, 1994). Here we demonstrated that

the presence of an intraguild predator altered the outcome of the indirect genetic effect and represents an IEE. IEEs can be important factors in determining the fitness of species within a community and can influence the ecological and evolutionary dynamics of interacting species (Astles et al., 2005; TerHorst et al., 2015). The impact of community genetic effects are highly context-specific and a greater understanding of the role of IGEs and IEEs is crucial to establishing how these interactions affect host trait variation and, in turn, how they shape host-parasite interactions (Ashbrook et al., 2015; Astles et al., 2005; Khudr et al., 2018b; Moore et al., 1997; Rowntree et al., 2011; Whitham et al., 2003). Although the effect of IGP was not significant in affecting mummy position in our analysis of the split family groups it was marginal, and considering the small sample size in this analysis, it warrants further investigation. The molecular basis of these indirect effects is still unknown and may provide further insights into the evolution of traits that affect the interactions of species within a community.

We did, however, establish that IGP is significant in reducing the virulence of the parasitoid on the N116 aphid, highlighting the importance of considering the interaction between species when designing effective biological control schemes (Ferguson and Stiling, 1996). Moreover, our findings have shown that the most important factor in predicting parasitoid virulence is the immunity in the aphid lineages. It represents the largest contributor to the explained variance, on the parasitoid virulence, across all our analyses. As previously mentioned, aphid resistance to parasitoids is predominantly attributed to the presence of defensive secondary symbionts (Kraft et al., 2017; Oliver et al., 2005). As these symbionts are vertically transmitted in clonal populations (Oliver et al., 2003), and reside inside the aphid host, untangling the effects of the symbionts and host is very difficult. However, it is important to note that two strains of resistant pea aphids, that do not carry any secondary symbionts, have been reported (Martinez et al., 2014b). This means that there is an additional unknown aphid-based mechanism of resistance to parasitoids in some populations that may contribute to the huge variation in levels of protection observed (Martinez et al., 2018; Martinez et al., 2014a).

In our analysis, any effect of the different symbionts, present in the two aphid populations, on wasp virulence and aphid behaviour would show as an effect of the aphid identity (or genotype). Moreover, the comparison of the N116 and Quad genotypes does show that there was a significant effect of the aphid genotype on

wasp virulence. When you consider the strong association between the presence of defensive symbionts and resistance to parasitoids in the pea aphid, and the lack of evidence for an endogenous defence in aphid linages that carry secondary symbionts (Kraft et al., 2017; Oliver et al., 2005), it is reasonable to assume that the significant effect of the aphid genotype in this analysis is caused by the presence of these symbionts. In the N116 aphid clone, we confirmed the presence of the secondary symbionts *H. defensa* and *F. symbiotica* that were previously reported in this clone (Kanvil et al., 2014) but also had one sequence match with another defensive symbiont *S. symbiotica*, that to my knowledge, has not been noted in this lineage before. Furthermore, we found only one secondary symbiont in the Quad aphid, *S. symbiotica.* Untangling the effect of the symbiont from the host is important because, unless we do, we cannot conclude for certain that this interaction represents an IEE as there is there is small possibility that the interaction is in fact a genotype effect rather than a true IEE, or a combination of both; and further work should look to eliminate that uncertainty.

The symbiosis, in this context, alters the outcome of the interaction between the parasitoid and the aphid host and should also be considered as an important indirect ecological effect (IEE) in this system (Wootton, 1994). Isolates of both *Serratia symbiotica* and *Hamiltonella defensa* have been shown to confer resistance to parasitoid wasps in the pea aphid, reducing successful parasitism by 23% and 42% accordingly (Oliver et al., 2005; Oliver et al., 2003). The occurrence of superinfected aphid clones, that carry multiple inherited symbionts, has been noted despite the apparent costs to aphid fecundity (Oliver et al., 2006). Aphids superinfected with *H. defensa* and *F. symbiotica* are known to have very high levels of resistance against *A. ervi*, up to 100% in some clones (Guay et al., 2009), and explains the high levels of resistance in the N116 clone. Moreover, aphids that were artificially inoculated with both *H. defensa* and *S. symbiotica* together showed increased resistance to parasitoids over the singularly infected aphids (Oliver et al., 2006). The presence of three defensive symbionts in the N116 aphid clone is unusual and further work is required to understand the significance of this finding.

The exact mechanisms by which the defensive symbionts provide the resistance to parasitoids is largely unknown. However, the aphid's association with *H. defensa* has received the most attention over the last decade. In the case of *H. defensa,* it is believed that the variation in the protection is caused by the presence or

absence of infection of the bacteria with different bacteriophages called APSEs. (Oliver et al., 2009). These bacteriophages are thought to encode putative toxins that function in the defence against parasitoids (Degnan and Moran, 2008; Oliver and Higashi, 2018). Aphids that carry particular *H. defensa* strains, but no phage, showed no protection against parasitoids whilst those that carried both the symbiont and one of four known APSEs showed different levels of parasitoid resistance, depending on the strains of each that was present (Brandt et al., 2017). The timing of the wasp mortality during development is also dependent on the strain of *H. defensa* and APSE present in the aphid (Brandt et al., 2017).

The defence offered by *H. defensa* to aphids is as highly specific as it is variable, the combinations of *H. defensa* and APSE that offer protection against *A. ervi* do not offer protection from other species of related parasitoid (Oliver and Higashi, 2018). Recent studies have also shown that the protection is also dependent on environmental conditions, with some strains no longer offering protection with relatively small increases of temperature (Doremus et al., 2018). Much of the literature suggests that there is a strong correlation between the protective phenotype of the aphid and the different *H. defensa*/APSE strains, and, when combined with the lack of evidence for the encapsulation of parasitoids in these linages of pea aphid, their immunity is likely to be dependent on the presence of defensive symbionts (Martinez et al., 2018). We have not identified the APSE present in the *H. defensa* found in the N116 aphid clone and further work is required in order to better understand how the interactions between these species shape the aphid phenotype.

Interestingly, The presence of *H. defensa* in a host aphid has further implications for the plant-aphid-parasitoid system as it also alters the behaviour of the parasitoids (Oliver and Higashi, 2018; Oliver et al., 2012). Parasitoid wasps alter the internal environment of the aphid, using both maternal and embryonic factors, to make it more favourable for wasp development (Oliver et al., 2012). The potential of *A. ervi* to differentiate between aphids infected with *H. defensa* and those that are not is demonstrated by an increased occurrence of superparasitism in the infected aphids. It is thought that by increasing the number of eggs in the aphid, the effect of the maternal and embryonic factors could also be increased (Oliver et al., 2012). Superparasitism occurs when more than one egg is deposited into the same aphid host and under normal conditions, this behaviour is usually considered to be maladaptive as it results in siblicide (Mackauer and Chau, 2001). Moreover,

superparasitism in uninfected aphids did not increase the success of parasitism, but it did in some of the infected aphid lines suggesting that it is adaptive under certain circumstances (Oliver et al., 2012). The wasp's ability to differentiate between the infected and uninfected aphids is thought to be the result of a decreased production of a major component of the aphid alarm pheromone, *trans*-β-farnesene (EBF) (Oliver et al., 2012). The alarm pheromone is secreted from cornicles when the aphids are attacked, and when aphids detect this pheromone they move away from the source, with some even dropping from the plant altogether (Oliver et al., 2012). Moreover, *H. defensa* is also implicated in attenuating the release of herbivore-induced plant volatiles that attract parasitoid wasps, further highlighting the importance of symbionts in the interactions between species (Frago et al., 2017; Oliver and Higashi, 2018)

This work has demonstrated the vastly complex nature of the interaction between the economically important agricultural pest *A. pisum* and its numerous interacting species. We further demonstrate the need to consider community genetic effects, genetic interactions between species and across trophic levels, in order to design more effective biological controls in agro-ecosystems.

12. Reproductive and phenotypic plasticity of an important aquatic model organism to multiple abiotic stressors

12.1 Abstract

Daphnia represent keystone species that underpin the normal functioning of the ecosystems in which they are found, they are also an important model system in the study of phenotypic plasticity and ecosystem health. With climate change and other anthropogenic influences affecting freshwater ecosystems at unprecedented levels, understanding how environmental stress effects populations of daphnia is critical to protecting biodiversity in freshwater ecosystems. Whilst we understand how stressors such as salinification and acidification of watercourses affect daphnia when they occur alone, we do not yet fully understand the effect of the more complex scenario of combined stressors and the long-term impact of them on *Daphnia* reproductive success and age structure. The types of stress that aquatic organisms now face are multifaceted and increases in salinity can occur with other factors such as acidification, light and chemical pollution and increased temperature, the ability of *Daphnia* to survive and reproduce in these scenarios, long term, is unknown. We exposed our laboratory raised lineage of *Daphnia magna* to different treatment levels of acidity, salinity, light stress (24-hour light/dark) and their combinations over 30 days. After 10 days under 24-hour light, the normal reproduction of the *Daphnia* populations was severely impeded compared to the controls. However, with the exception of the higher acidity treatments, there were still tangible increases in population size compared to their starting population. Moreover, across all treatments on day 30 the reproductive success dramatically increased, suggesting a plastic response in daphnia tolerance to the treatment conditions. Under 24-hour darkness large mortality occurred across all treatments, indicating that the constant absence of light had a much greater negative impact than the constant exposure to light. Overall, the population age structure varied dramatically in the absence of normal photoperiod suggesting that the life history responses in this scenario were context specific.

Our findings expand our understanding of the ecology of an economically important aquatic crustacean under complex abiotic environmental stress. This work furthers our understanding of the ability of aquatic organisms to adapt to the novel environments created by the various anthropogenic changes to ecosystems.

12.2 Introduction

Global warming and anthropogenic influences on aquatic ecosystems have increased the effects and occurrence of tidal intrusions of seawater, storm surges and acidification (via the burning of fossil fuels, mining, dredging of waterways, agricultural and industrial processes, etc) of freshwater ecosystems (Hall and Burns, 2002; Weber and Pirow, 2009). These environmental stressors have profound effects on the structure of freshwater communities. *Daphnia* is a keystone zooplankton and an important model in the study of environmental stress and loss of biodiversity in freshwater ecosystems (Gannon and Stemberger, 1978). Furthermore, *Daphnia* are widely used to evaluate toxicity in aquatic environments (Jansen et al., 2015) and their ability to tolerate a range of abiotic stressors, including elevated salinity (Schuytema et al., 1997), has been crucial to their application in tolerance studies for over 25 years (Latta et al., 2012).

The genome of *Daphnia* may express different phenotypes in reaction to different environmental conditions; a phenomenon referred to as phenotypic plasticity (West-Eberhard, 2003). On the one hand, salinity and acidity pose a major challenge for Cladocerans in their habitats around the globe. *Daphnia magna* is a generalist cladoceran whose niche is defined by a much higher range of salinity tolerance than other aquatic *Daphnia* species, and populations of *D. magna* have been documented to inhabit both fresh and brackish waters (Teschner, 1995), with strong evidence of their ability to vary their osmoregulation tactics to cope with varying levels of salinity (Martínez-Jerónimo and Martínez-Jerónimo, 2007). Moreover, the acidification of fresh water ecosystems has been shown to alter the structure of Cladoceran populations and reduce the species richness of zooplankton (Locke and Sprules, 2000; Pollard et al., 2003). *Daphnia* species are less abundant in acidified lakes and the numbers of other non-daphnid species (cladocerans, calanoid copepods, and insects) increase with some even dominating the ecosystems (Brönmark and Hansson, 2017; Weber and Pirow, 2009). The combined effects of

salinity and acidity pose another major challenge in aquatic ecosystems. It is becoming more common for the bodies of water, that contain *Daphnia*, to show increased salinity and acidity due to various natural and anthropogenic effects thus making the conditions of the microhabitats more hostile and detrimental to life (Altshuler et al., 2011).

Light is another important stimulus to *Daphnia*, shorter days with less light is an environmental cue that induces sexual morphs and the production of diapausing eggs (Toyota et al., 2018), and light is also essential for the hatching of dormant eggs (Shan, 1970; Vandekerkhove et al., 2005). However, little is known about the effects of an absence of photoperiod, *i.e.* the continuous exposure to light or continuous absence of light, on *Daphnia* fitness and survival. This can occur in areas with high levels of light pollution, eutrophic environments with low light incidence, and in areas, close to the earth's polar circles, that experience both extended periods of polar night and midnight sun. Much of the light exposure research on *Daphnia* and other arthropods has focused its interest on the circadian clock and rhythm and the diel vertical migration of zooplankton in response to variations in photoperiod.
The salinization of freshwaters has been shown to affect the abundance and diversity of zooplankton and can even allow new species to colonise areas outside of their natural range (Heine-Fuster et al., 2010). The negative effects of salinization on freshwater zooplankton results from their limited osmoregulatory capability, a response that can affect all aspects of their biology, from feeding rate to reproduction and growth rate (Achuthankutty et al., 2000). Species of *Daphnia*, including *D. magna*, display an osmoconformer response to increases in salinity, a response that is found across marine crustaceans (Heine-Fuster et al., 2010). Moreover, some species, such as *D. exilis* and *D. Pulex*, can maintain a positive and constant osmolality difference with the environment at higher salinities via an osmoregulatory response (Heine-Fuster et al., 2010). Osmoregulatory responses are associated with a high energetic cost but may allow certain *Daphnia* to colonise environments that other species cannot (Heine-Fuster et al., 2010).

The clonal populations of *D. magna* used in laboratory experiments show a range of salinity tolerance from fresh water to brackish (10g L^{-1}) with the upper limit of their tolerance changing depending on where the population was sampled from across their natural range (Aladin and Potts, 1995). The different clones of *Daphnia* display quantitatively measurable differences in sodium uptake kinetics (Havas et al.,

1984; Potts and Fryer, 1979), suggesting that tolerance and osmoregulation are genotype specific. The effect of temperature on *Daphnia's* ability to osmoregulate is complex and the physiological process that mediates osmoregulators is largely unknown. *D. magna* shows a wider tolerance to salinity with increases in temperature as a result of an increase in osmoregulatory ability (Aladin and Potts, 1995). Moreover, osmoregulation is also pH dependent, no cladocerans have been found in waters with high pHs, such as in soap lakes (pH 9.8), where other anostracans, such as *Branchinecta lindhli*, have been found (Aladin and Potts, 1995; Prophet, 1963). In contrast, some cladocerans thrive at low pH levels, low pH is thought to inhibit sodium uptake in daphnia much as it does in other freshwater fish (Aladin and Potts, 1995).

To date, information on the effect of a lack of photoperiod, especially when combined with other environmental stressors (*e.g.* salinity and acidity), is surprisingly sparse, and despite fresh water salinization and acidification being extensively documented we do not yet fully understand the effects of the more complex scenarios of combined stressors on the population dynamics of aquatic organisms under constant light or darkness (Degens, 2013; Kaushal et al., 2018; Zalizniak et al., 2009). It has been shown that the negative effects of too little or too much light extends beyond the direct effect on circadian rhythms and can negatively impact the survivorship of *Daphnia parvula* after as little as 7 days of exposure (Connelly et al., 2016). The effect of dark conditions varies between clones, with the clones that showed reduced fecundity also showing greater survivorship by an unknown mechanism (Connelly et al., 2016). The importance of light in the plastic response of *D. magna* to predatory fish kairomones had been overlooked for decades and more recently it was shown that *D. magna* does not respond to the presence of kairomones, in the absence of light (Effertz and von Elert, 2014). Many species of *Daphnia* show huge variation in various stress tolerance traits and one species, *Daphnia parvula*, has demonstrated that the tolerance to constant dark similar clonal variation (Connelly et al., 2016).

Another aspect of exposure to sunlight, which is more comprehensively studied, is the effect of UV radiation. It is well understood that exposure to UVR causes DNA damage and can act as a physiological stress in some organisms. More recently there has been some evidence suggesting that there are some positive effects of UVR

exposure such as reducing pathogen and parasite populations within ecosystems (Connelly et al., 2016).

In this work, we have exposed a clone of *Daphnia magna* to different stressors, salinity, acidity and absence of a photoperiod (constant exposure to light or dark conditions for 30 days), or a combination of the stressors to explore a new area of *Daphnia* tolerance and raise the following questions:

1. How does *Daphnia magna* reproductive success differ across salinity and acidity and their combinations under constant light exposure?
2. How does reproductive success vary under continuous light versus constant darkness subject to the effects of salinity and acidity stressors?
3. How do the combinations of stressors affect *Daphnia magna* age structure?

12.3 Methods

12.3.1 Study organism: *Daphnia magna*

The clonal population of *Daphnia magna* (Straus) used in this experiment was established from a single female sampled from a population purchased from Sciento ©, Manchester UK. The resulting clonal population were reared in a growth chamber, at the Faculty of Biology, Medicine and Health, The University of Manchester, under artificial light with a 16:8 light cycle and at a temperature of 23°C. The *Daphnia* were maintained on a diet of 1ml of baker's yeast (*Saccharomyces cerevisiae*) and 2ml of alga *Scenedesmus quadricauda* (fed every 2nd day) in a modified version (Ebert, 2013a) of Artificial *Daphnia* Medium (ADaM) (Klüttgen et al., 1994) at pH 7 and 0.33g L^{-1}salinity (as outlined in section 10.2).

12.3.2 Set up and experimental design

During the experiment, we artificially manipulated the salinity and acidity of the ADaM in order to understand how these natural environmental stress conditions affect *Daphnia* fitness and age structure. The conditions were based on extensive pilot studies where we established the different levels of pH and salinity that were sublethal to our clone of *D. magna* for long exposures. The experimental treatments were as follows:

The pH treatments were:

1. pH 6.
2. pH 55.
3. pH 5.

The salinity treatments (plus ADaM salinity) were:

1. 1.33 g L^{-1}.
2. 3.33 g L^{-1}.
3. 6.33 g L^{-1}.

The combined stress treatment conditions were:

1. 1.33g L^{-1} & pH 6.
2. 3.33g L^{-1} & pH 5.5.
3. 6.33g L^{-1} & pH 5.

Each of these nine treatment conditions was then carried out with photoperiod treatments of either 24h dark or 24h light, making a total of 18 possible treatments. The positive control was the standard ADaM condition in 24-hour light or dark and the negative control was the standard ADaM condition in the optimal photoperiod of 16:8. We used Sigma Aldrich 'sea salts' and distilled acetic acid (Sarson's ©), procured from a local supplier, for all treatments. To lower the pH in the treatment conditions the acetic acid was added dropwise to the beaker of media and measured continuously using a 'Mettler Toledo™ FE20 FiveEasy™ benchtop pH meter' until the desired pH was reached. The salinity conditions were made by simply dissolving the correct weight of sea salts in the beaker. All the experimental beakers were kept in an experimental growth chamber with a temperature of 23°C.

Each condition and control were tested in beakers containing 600ml of ADaM media made up to the relevant experimental conditions prior to the addition of seven *Daphnia* nymphs, with each treatment and control being repeated three times. The 24-hour dark treatment group were kept in containers that had been wrapped in foil to ensure complete darkness within the microcosm. A 75% media change was conducted weekly, in the light exposed daphnia only, to prevent the build-up of harmful metabolites, however, to eliminate any exposure to light the 24-hour dark treatment groups the media was not changed. The location of the daphnia microcosms, within the controlled environment chamber, were randomised when

they were fed (every 2nd day). The data was collected on day 10 and day 30 in the 24-hour light treatment and on day 30 for the 24-hour dark treatment. However, due to the high mortality under specific combinations, not all treatments and repeats survived to the 30-day final data collection and these were omitted from the analysis. The final number of treatments used in the analyses was nine, and with the controls and repeats, a total of 32 individual beakers was used per light treatment (24h light & 24h dark). During the two data collections, we counted the total number of *Daphnia* and the number of juveniles and adults.

12.3.3 Statistical analysis

12.3.3.1 24-hour light

All statistical analyses were conducted in R (R Core team, 2017) via R studio (RStudio Team, 2015). A generalised mixed effects model (GLMM), family 'Poisson', model optimiser 'bobyqa' (Powell, 2009), R packages 'lme4' (Bates et al., 2015) and 'car' (Fox and Weisberg, 2011), was applied to test *Daphnia* reproductive success throughout the experiment duration (GLMM 1). The census (count day) was randomised. This was followed by a posteriori test (Tukey) to examine multiple pairwise comparisons of the stressors in question.

The explanatory variable was the salinity or acidity treatment under 24h light comprising the following levels:

- Baseline = *Daphnia* in ADaM with optimal salinity 0.33 g L^{-1}, acidity pH 7 and under 24 hour light.
- Salinity:
  - Level 1= 1.33g L^{-1}.
  - Level 2= 3.33g L^{-1}.
  - Level 3= 6.33g L^{-1}.
- pH:
  - Level 1= pH 6.
  - Level 2= pH 5.5.
  - Level 3= pH 5.
- Combined:
  - Salinity stress level 1 + acidity level 1.
  - Salinity stress level 2 + acidity level 2

        - Salinity stress level 3 + acidity stress level 3.
    - The negative control = *Daphnia* in ADaM with optimal salinity 0.33g $L^{-1}$and acidity pH 7, with 16:8h photoperiod was used as a frame of reference.

Another GLMM (GLMM 2) was used to analyse age structure with the same set up as above but with the response variable being the ratio of juveniles to adults per beaker per census.

12.3.3.2 24-hour dark

The structure of the analysis is as explained in the 24-hour light. However, the data collection for this part of the experiment took place only at day 30 as we could not count the *Daphnia* without exposing them to light and it was essential to eliminate all exposure to light during this part of the experiment. The same data was collected: the total number of *Daphnia* and number of juveniles & adults. As so few of the Daphnids were able to survive to the 30-day data collection we were unable to conduct any statistical analysis. However, some descriptive comparisons with the results of the 24-hour light treatments have been drawn.

12.4 Results

Under constant exposure to light, i.e. lack of the normal photo period, the *Daphnia* population size was significantly affected by the stress treatments (X^2 = 1428.6, Df = 9, P <0.0001). Specifically, all levels of stress had a differential highly significant influence on *Daphnia* reproductive success; see Figure 30 and Table 26 for effects details. See also Table 27 for *a posteriori* multiple pairwise comparisons of the stress levels.

When comparing the positive and negative controls we can see the effects of the 24-hour light stress alone. The population size of the negative control had increased by 1236% from day one to day ten whereas, the positive control (under constant light) the population had only increased by 286%. After 30 days the population size had increased a further 11% in the negative control and 604% in the positve control. Moreover, after 30 days the daphnia under constant light showed increased repoductive success over those in a normal photo period, their average population size on day 30 (190 indaviduals) was almost double that of the daphnia in

the normal photo period (103.5 indaviduals). This would suggest that the daphnia experienced delayed repoduction under 24-hour light, but were able to aclimatise to it after multiple generations.

Overall, under constant light, the ratio of ratio juvenile *Daphnia* to adults was significantly affected by the stress treatment under constant light (X^2 = 124.96, Df = 9, P <0.0001). Specifically, all levels of stress had a differential highly significant influence on Daphnia reproductive success; see Table 28 for effects details. See also Table 29 for *a posteri ori* multiple pairwise comparisons of the stress levels. The average proportion of adults in the negative control increased from 14% (13.0 indaviduals) on day 10 to 33% (34.2 indaviduals) on day 30 and the in the positive control (under 24-hour light) it fell from 31% (8.4 indviduals) to 5% (9.5 indaviduals) suggesting that the daphnia in the 24 hour light stress experienced delayed maturation in responce to the absence of photoperiod.

Moreover, across all treatments under constant light, the number of *Daphnia* at the day 10 count is significantly reduced, when compared to the negative control (average percentage increase/decrease in population size on day 10 compared to day 1: -ve control= +1236% salinity level 1= +485%, level 2= +48% and level 3= +279%. pH level 1= +176%, level 2= -29% and level 3= -64%. Salinity level 1 + acidity level 1= +326%, salinity level 2 + acidity level 2= +67%, salinity level 3 + acidity level 3= +52%). However, from day 10 to day 30, and across all the treatments, the average population sizes increased dramatically when compared to the negative control during this period, again suggesting that our *Daphnia magna* clone was able to aclimatise to the treatment conditions and delayed their repoduction in response to the treatment conditions (average percentage increase in population size on day 30 compared to day 10: -ve control= +11%, +ve control= +604%, salinity level 1= +144%, level 2= +965% and level 3= +921%. pH level 1= +171%, level 2= +1865% and level 3= +1030%. Salinity level 1 + acidity level 1= +200%, salinity level 2 + acidity level 2= +2400%, salinity level 3 + acidity level 3= +319%).

Table 27 also shows that the acid treatments had a greater negative impact on *Daphnia* repoductive success than the salinity treatments. Interestingly, the *Daphnia* in the combined acidity/salinity treatments actually perfomed better than in the acid treatment alone. After 30 days four treatments under light stress had a greater repoductive success than the negative control (ADaM, Sal 2, Sal 3 and Sal 2/Acid 2)

and in each of these the proportion of adults was also reduced from 33% in the negative control to 5%, 20%, 10% and 16%, respecively. Under the same treatments but in constant darkness so few daphnia were unable to survive to the day 30 count that we were unable to make any statistical analysis. This would imply that the stressed induced by constant darkness has a much greater effect on *Daphnia* fitness.

Of the 28 replicates (9 salinity & acidity treatments × 3 repeats of each + positive control) of *Daphnia magna* that were exposed to the same salinity and acidity treatments but under 24-hour darkness, only 5 survived to the 30-day census. Moreover, the salinity level 1 treatment was the only treatment in the 24-hour darkness treatment where all three replicates survived and their population at day 30 increased by only 43%. In the salinity + acidity 1 and acidity 1 treatments only one replicate survived to day 30 with their population changes being +48% and -95% respectively. From this, we can infer that the 24-hour dark has a much greater negative impact on daphnia reproductive success than the 24-hour light treatments.

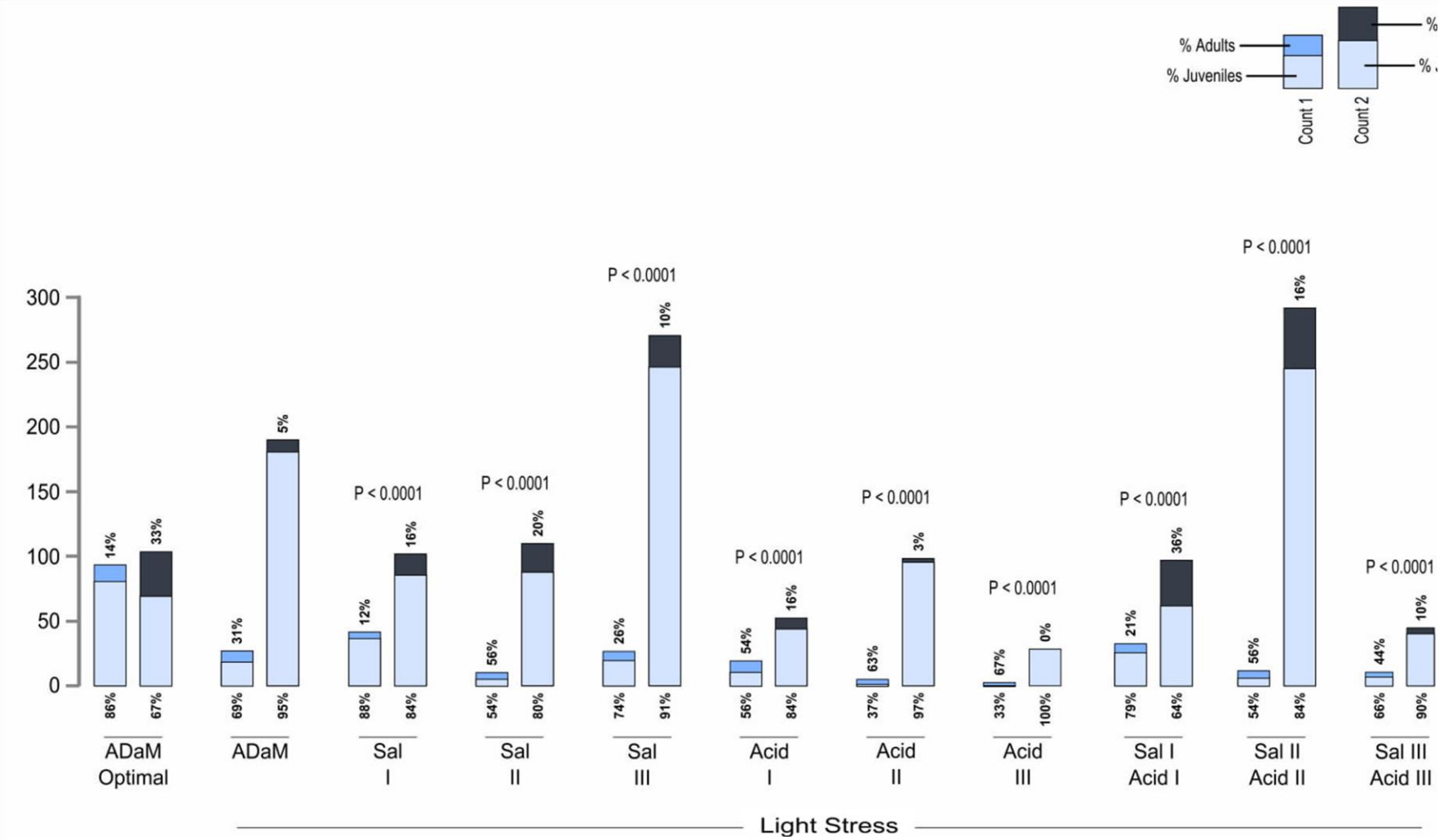


Figure 30: *Daphnia* population sizes and age structure at day 10 and 30

The *Daphnia* population size and age structure across all treatments at count 1 (day 10) and count 2 (day 30). The conditions for each treatment are as follows: ADaM optimal- negative control, standard media conditions with a photoperiod of 16:8. ADaM light stress- positive control, standard media conditions with 24-hour light. Light stress (24-hour light) with salinity (Sal) level 1- 1.33 g L^{-1}, level 2 - 3.33 g L^{-1} and level 3 - 6.33 g L^{-1}. Acidity (Acid) level 1- pH 6, level 2- pH 5.5 and level 3- pH 5

Table 26: GLMM 1 summary
Effects of the different levels of the stress, under constant light, on *Daphnia* reproductive success taken from GLMM1, specified in the Methods are displayed. Salinity level 1- 1.33 g L^{-1}, level 2 - 3.33 g L^{-1} and level 3 - 6.33 g L^{-1}. pH level 1- pH 6, level 2- pH 5.5 and level 3- pH 5

Fixed effects:				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	4.28275	0.68123	6.287	<0.0001
Light stress (Salinity 1)	-0.41472	0.06803	-6.096	<0.0001
Light stress (Salinity 2)	-0.58963	0.07122	-8.278	<0.0001
Light stress (Salinity 3)	0.31384	0.06314	4.970	<0.0001
Light stress (Acidity 1)	-1.10787	0.08339	-13.286	<0.0001
Light stress (Acidity 2)	-0.74274	0.06873	-10.806	<0.0001
Light stress (Acidity 3)	-1.95401	0.10214	-19.132	<0.0001
Light stress (Salinity 1) + (Acidity 1)	-0.51750	0.06986	-7.408	<0.0001
Light stress (Salinity 2) + (Acidity 2)	0.33494	0.05833	5.742	<0.0001
Light stress (Salinity 3) + (Acidity 3)	-1.36652	0.09125	-14.976	<0.0001

Table 27: A posteriori comparison 1
Multiple pairwise comparisons as results of the posthoc Tukey test, following GLMM1 examining *Daphnia* reproductive success are displayed. Only significant results are shown. Salinity level 1- 1.33g L^{-1}, level 2 - 3.33g L^{-1} and level 3 - 6.33g L^{-1}. pH level 1- pH 6, level 2- pH 5.5 and level 3- pH 5

Linear hypotheses:				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Salinity 1 vs Control== 0	-0.41472	0.06803	-6.096	<0.001
Salinity 2 vs Control == 0	-0.58963	0.07122	-8.278	<0.001
Salinity 3 vs Control == 0	0.31384	0.06314	4.970	<0.001
Acidity 1 vs Control == 0	-1.10787	0.08339	-13.286	<0.001
Acidity 2 vs Control == 0	-0.74274	0.06873	-10.806	<0.001
Acidity 3 vs Control == 0	-1.95401	0.10214	-19.132	<0.001
Salinity 1 + Acidity 1 vs Control == 0	-0.51750	0.06986	-7.408	<0.001
Salinity 2 + Acidity 2 vs Control == 0	0.33494	0.05833	5.742	<0.001
Salinity 3 + Acidity 3 vs Control == 0	-1.36652	0.09125	-14.976	<0.001
Salinity 3 vs Salinity 1 == 0	0.72856	0.06331	11.508	<0.001
Acidity 1 vs Salinity 1 == 0	-0.69315	0.08352	-8.300	<0.001
Acidity 2 vs Salinity 1 == 0	-0.32802	0.06889	-4.762	<0.001
Acidity 3 vs Salinity 1 == 0	-1.53928	0.10224	-15.056	<0.001
Salinity 2 + Acidity 2 vs Salinity 1 == 0	0.74966	0.05851	12.812	<0.001
Salinity 3 + Acidity 3 vs Salinity 1 == 0	-0.95180	0.09137	-10.417	<0.001
Salinity 3 vs Salinity 2 == 0	0.90347	0.06673	13.540	<0.001

Acidity 1 vs Salinity 2 == 0	-0.51824	0.08614	-6.017	<0.001
Acidity 3 vs Salinity 2 == 0	-1.36438	0.10439	-13.070	<0.001
Salinity 2 + Acidity 2 vs Salinity 2 == 0	0.92457	0.06219	14.866	<0.001
Salinity 3 + Acidity 3 vs Salinity 2 == 0	-0.77689	0.09377	-8.285	<0.001
Acidity 1 vs Salinity 3 == 0	-1.42171	0.07958	-17.865	<0.001
Acidity 2 vs Salinity 3 == 0	-1.05658	0.06406	-16.493	<0.001
Acidity 3 vs Salinity 3 == 0	-2.26784	0.09905	-22.896	<0.001
Salinity 1 + Acidity 1 vs Salinity 3 == 0	-0.83134	0.06527	-12.738	<0.001
Salinity 3 + Acidity 3 vs Salinity 3 == 0	-1.68036	0.08778	-19.142	<0.001
Acidity 2 vs Acidity 1 == 0	0.36513	0.08409	4.342	<0.001
Acidity 3 vs Acidity 1 == 0	-0.84614	0.11304	-7.485	<0.001
Salinity 1 + Acidity 1 vs Acidity 1 == 0	0.59037	0.08501	6.945	<0.001
Salinity 2 + Acidity 2 vs Acidity 1 == 0	1.44281	0.07582	19.029	<0.001
Acidity 3 vs Acidity 2 == 0	-1.21126	0.10271	-11.793	<0.001
Salinity 1 + Acidity 1 vs Acidity 2 == 0	0.22524	0.07069	3.186	0.0432
Salinity 2 + Acidity 2 vs Acidity 2 == 0	1.07768	0.05932	18.166	<0.001
Salinity 3 + Acidity 3 vs Acidity 2 == 0	-0.62378	0.07069	-6.788	<0.001
Salinity 1 + Acidity 1 vs Acidity 3 == 0	1.43650	0.05932	13.884	<0.001
Salinity 2 + Acidity 2 vs Acidity 3 == 0	2.28894	0.09606	23.829	<0.001
Salinity 3 + Acidity 3 vs Acidity 3 == 0	0.58749	0.11896	4.939	<0.001
Salinity 2 + Acidity 2 vs Salinity 1 + Acidity 1 == 0	0.85244	0.06062	14.061	<0.001

Table 28: GLMM 2 summary
Effects of the different levels of the stress, under constant light, on *Daphnia* age structure taken from GLMM 2, specified in the Methods. Salinity level 1- 1.33 g L^{-1}, level 2 - 3.33 g L^{-1} and level 3 - 6.33 g L^{-1}. pH level 1- pH 6, level 2- pH 5.5 and level 3- pH 5

Fixed effects:				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	4.399638	0.100751	43.669	<0.0001
Light stress (Salinity 2)	-0.202026	0.074397	-2.716	0.007
Light stress (Acidity 1)	-0.184496	0.077863	-2.369	0.018
Light stress (Acidity 2)	-0.272326	0.079613	-3.421	0.0006
Light stress (Acidity 3)	-1.261128	0.134183	-9.399	<0.0001
Light stress (Salinity 1) + (Acidity 1)	-0.139355	0.073374	-1.899	0.058
Light stress (Salinity 2) + (Acidity 2)	-0.167793	0.073832	-2.273	0.023

Table 29: A posteriori comparison 2

Multiple pairwise comparisons as results of the posthoc Tukey test, following GLMM2 examining *Daphnia* population age structure. Only significant results are shown. Salinity level 1- 1.33 g L^{-1}, level 2 - 3.33 g L^{-1} and level 3 - 6.33 g L^{-1}. pH level 1- pH 6, level 2- pH 5.5 and level 3- pH 5

Linear hypotheses:				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Acidity 2 vs Control == 0	-0.272326	0.079613	-3.421	0.021
Acidity 3 vs Control == 0	-1.261128	0.134183	-9.399	<0.01
Salinity 2 vs Salinity 1 == 0	-0.251591	0.066488	-3.784	<0.01
Acidity 1 vs Salinity 1 == 0	-0.234060	0.070345	-3.327	0.028
Acidity 2 vs Salinity 1 == 0	-0.321891	0.072277	-4.454	<0.01
Acidity 3 vs Salinity 1 == 0	-1.310693	0.129965	-10.085	<0.01
Salinity 2 + Acidity 2 vs Salinity 1 == 0	-0.217358	0.065855	-3.301	0.031
Acidity 3 vs Salinity 2 == 0	-1.059102	0.132076	-8.019	<0.01
Acidity 2 vs Salinity 3 == 0	-0.275371	0.079555	-3.461	0.018
Acidity 3 vs Salinity 3 == 0	-1.264173	0.134149	-9.424	<0.01
Acidity 3 vs Acidity 1 == 0	-1.076632	0.133901	-8.041	<0.01
Acidity 3 vs Acidity 2 == 0	-0.988802	0.134926	-7.328	<0.01
Salinity 1 + Acidity 1 vs Acidity 3 == 0	1.121773	0.131503	8.530	<0.01
Salinity 2 + Acidity 2 vs Acidity 3 == 0	1.093335	0.131759	8.298	<0.01
Salinity 3 + Acidity 3 vs Acidity 3 == 0	1.207908	0.134803	8.961	<0.01

12.5 Discussion

We have demonstrated that in our daphnia magna linage, exposure to light stress alone reduced reproductive success and altered the age structure of the population. With constant darkness the daphnia were unable to survive and with constant light, the daphnia seem to be able to acclimatise to the stress and after 30 days the population size in the positive control was 84% greater than the negative control (at day 30) and represented a 604% increase over the 10-day population size. However, the proportion of adults is reduced, and as after 10 days there is a similar number of adults in the 24 hour light it is likely as result of a shift in life-history traits in response to stress, an adaptive response that has been noticed in association with food stress, presence of predators and as a result of various chemical pollutants (Ashforth and Yan, 2008; Bodar et al., 1988; Coors et al., 2004; De Coen and Janssen, 1997; Dodson and Hanazato, 1995; Frost et al., 2010; Walton et al., 1982).

With the exposure to acidity or salinity and constant light, the response of the daphnia follows a similar pattern. They were able to acclimatise to the stress with population sizes increasing in all treatments from the 10-day to the 30-day census. The age structure of the populations in each of the treatments was highly variable suggesting that the daphnia's response, in each case, was dependant on the levels or type of stress that they encountered. However, more work is required to understand the exact nature of the context-specific changes in life history traits in terms of the underlying biochemical and physiological mechanisms.

Daphnia are known to produce diapausing eggs during periods of environmental stress, such as salinity, photoperiod, temperature, other seasonal effects and anthropogenic effects. The diapause of resting eggs enables the recovery of populations when the conditions are more favourable (Paes et al., 2016; Weider et al., 1997). The unfavourable conditions created by our stress conditions could have induced the production of resting eggs in some of the *Daphnia* in our experiment. Moreover, the production of diapausing resting eggs could account for the reduced growth of the *daphnia* populations during the first 10 days but is an area outside the parameters of this experiment and one that requires further enquiry.

Low pH levels have many different effects on *Daphnia magna*, including reduced survival and reductions in growth (Alibone and Fair, 1981; El-Deep Ghazy et al., 2011). When *D. magna* is exposed to naturally occurring freshwater with a pH

as low as 3.31, all individuals died within a few hours (Alibone and Fair, 1981). Furthermore, changes in water pH can also be attributed to the effect of acid rain and acid spills (El-Deep Ghazy et al., 2011). The levels of mortality seen as the pH is reduced is attributed to a sharp decline in respiratory rates. In water, carbon dioxide exists in three possible forms and the pH of the water determines the proportions in which they are found, as the pH decreases the quantity of carbon dioxide in the 'CO_2- free' form increases (Alibone and Fair, 1981). At pH 6 the level of free CO_2 is more than 60% and at pH 4 this increases to 100%. Furthermore, Alibone and Fair (1981) believe the higher concentration of CO_2 is sufficient to inhibit the diffusion of CO_2 from the *Daphnia* gills. The increased concentration of CO_2 within the daphnia would subsequently increase the pH of the *daphnia* haemolymph thus reducing its affinity for oxygen, in accordance with the Bohr effect, and reduce the uptake of oxygen (Alibone and Fair, 1981). This phenomenon is believed to be crucial to understanding the tolerance of aquatic organisms to acidic environmental conditions (Alibone and Fair, 1981).

The survival of *daphnia* in environments with high salinity depends on their halotolerance. The ability of *Daphnia* to regulate their osmolarity in the face of external ionic challenge is a result of their remarkable capacity to switch their osmoregulatory strategy between osmoconformance and osmoregulation (Heine-Fuster et al., 2010; Khudr et al., 2018a; Weider et al., 1997). In our day 30 data, the *D. magna* in the highest salinity level (6.33 g L^{-1}) showed increased fertility over those at lower salinities. In contrast, Heine-Fuster et al (2010) found that at salinities up to 6g L^{-1} and over short and long-term exposures, *D. exilis*, does not experience any changes in its population growth rate, but at a salinity of 8g L^{-1} or more, the fertility of the population is reduced and development is delayed. These negative effects were attributed, at least in part, to the higher energetic costs of maintaining an osmolality gradient at higher environmental salinities (Heine-Fuster et al., 2010).

Daphnia Pulex, like *D. magna*, populations naturally occur across environments with varying salinities and exist as genotypes that are genetically differentiated for their salinity tolerance (Latta et al., 2012; Smolders et al., 2005). Increases in salinity cause two problems for aquatic organisms, osmotic stress and ion cytotoxicity (Latta et al., 2012). Osmotic stress occurs when the salt concentration of the environment is higher than the intracellular salt concentration and as a result, water moves out of the cells. In response to osmotic stress, organisms

upregulate genes associated with the synthesis of osmoprotectants that stabilise proteins and cellular structures and increase osmotic pressure (Latta et al., 2012). Salt tolerant *D. pulex* genotypes have undergone regulatory evolution in the pathways that synthesise osmoprotectants and increase their tolerance to osmotic stress (Latta et al., 2012). Cytotoxicity refers to the disruption of the hydrophobic and electrostatic forces that maintain the structure of proteins and can inhibit the normal functioning of enzymes (Latta et al., 2012). Ion transport proteins are involved in the adaptive response to the cytotoxicity caused by salinity stress in daphnia and other crustaceans (Latta et al., 2012). The expression of, and gene activity in, the proteins involved in the transport of sodium and potassium ions is increased in response to increasing salinity, modifications of this pathway enable some genotypes of daphnia to tolerate higher levels of salinity (Latta et al., 2012).

The rates of sodium turnover in *daphnia* are high when compared to those of other freshwater animals but this is proportional when considering their small size and greater surface area to volume ratio (Potts and Fryer, 1979). The uptake of sodium in *D. magna* varies dramatically when comparing different geographically isolated populations and like many other traits in *Daphnia*, a degree of local adaptation is expected (Potts and Fryer, 1979). The uptake of sodium is reduced in acid conditions, this is likely as sodium uptake takes place in exchange for metabolically produced hydrogen ions (Potts and Fryer, 1979). Moreover, Potts and Fryer (1979) found that the rate of sodium loss, in their *Daphnia magna* isolates, increased fourfold between pH7 and pH3, they assert that the increased loss and reduced uptake of sodium could lead to death by sodium loss, especially when the concentrations of environmental sodium were low. This could offer an explanation as to why the *D. magna* in the salinity 2 & acidity 2 treatment outperformed the salinity 1 & acidity 1. In order to establish this, measurements of the uptake and efflux of haemolymph sodium would need to be measured across the treatment groups. Moreover, it implies that the effects of combined stressors when they occur synchronously, such as the acidification and salinization of freshwater, may exist in a state of precarious equilibrium and their interactions need to be considered carefully when trying to restore freshwater ecosystems and protect biodiversity.

The extent to which freshwater ecosystems are now exposed to salinity stress is unprecedented and whilst the effects of salinity on *Daphnia* under laboratory conditions is well studied, the ever-increasing large scale impact of this, on

freshwater ecosystems, is an understudied area given the scale of natural and anthropogenic changes around the globe (Cañedo-Argüelles et al., 2013; Coldsnow et al., 2017; Kefford et al., 2016). The effects of climate change and other anthropogenic activities are not, however, limited to increases in salinity as the combined effects of salinity with other factors such as the acidification of watercourses, pollution (light and chemical) and increases in global temperatures represents a lesser understood area of research. Understanding the effects of combined environmental stressors and their impact on the behaviour and reproductive success of a keystone species could be central to the protection of biodiversity in freshwater ecosystems.

Whilst there has been extensive research on the ability of *Daphnia* to survive various types of stress, there is comparatively little known about the effect of combined stressors. With the onset of global climate change and other anthropogenic changes to freshwater ecosystems, the ability of animals to avoid environmental stress is likely to be reduced and animals must rather adapt to them. In the unstable environments that are created an organism's ability to survive and reproduce under environmental stress depends on their levels of tolerance to these conditions, highlighting the importance of an understanding of this tolerance especially in keystone species. Moreover, further studies should help to understand the extent of interspecific variation in these responses and improve the understanding of the more complex scenarios caused by anthropogenic pressures that organisms face globally.

13. General discussion

In this thesis, I have sought to understand how variations in the biotic and abiotic factors of ecosystems can influence the plastic responses of aquatic and terrestrial arthropods. Here, I will discuss the main findings, some of the limitations of the approaches and suggest areas for further research.

In the aphid chapter I showed how the outcome of an established interspecific indirect genetic effect (IIGE) is altered by various factors and, most notably, how other community genetic effects (CG), such as indirect ecological effects (IEE) have a context-specific effect on the outcome of the interaction between species, within a community. The main findings of this chapter are as follows: 1) The interactions between species are context-dependent and will change depending on the specific genotypes of the species involved; 2) The introduction of an intraguild predator represents an IEE, that has the potential to alter the outcome of the interaction between the pea aphid and its parasitoid (*A. ervi*); 3) The aphid's association with various defensive symbionts is also context-specific and represents a particularly potent IEE that, in some cases, can reduce the parasitoid's reproductive success to zero. These findings highlight the importance of integrating community genetics into biological disease- and pest-control in order to maximise their effectiveness in controlling target species.

The high levels of immunity in the N116 aphid meant that we struggled to establish the quantitative genetic design in the way that we wanted. Of the approximately 150 sire × dam matings only 10 produced sufficient daughters to enable a split between experimental treatments of +IGP and −IGP, which may have contributed to the study only finding marginal effects. To really understand the effect of IGP, on the interaction between the parasitoid and aphid, I would suggest that the experiment should be repeated across multiple genotypes of aphid and with other intraguild predators (such as ladybird larvae). This will enable a better understanding of the role of genetic variation in the aphid, wasp and IGP on the outcome the interactions in this system. Another limitation to consider is that the method used entails randomly sampling bacterial colonies containing the plasmid with the inserted 16s gene sequence, and thus and how representative the sample is dependent on the number of colonies sampled. The method we used can therefore only be used to determine the presence of the symbionts sampled, rather than giving any insight to

the potential absence or presence of other symbionts and could mean that other symbionts remain to be discovered.

The second chapter, with *Daphnia magna*, showed how the various anthropogenic changes to the abiotic factors of aquatic environments can alter various behavioural and developmental plastic responses of this keystone species. We demonstrated that under the various combinations of stressors (light, darkness, salinity, acidity) the response of the daphnia was highly context specific. Moreover, in the short term the ability of the daphnia populations to reproduce under stress was severely impeded but after multiple generations they appeared, in most cases, to acclimatise to the new conditions. These findings further our understanding of the ecology of *Daphnia magna* under complex abiotic environmental stress and highlight their ability to adapt to the novel environments created by anthropogenic effects.

The main limitation of this experiment is that it utilised just one clonal population of *Daphnia magna*. The responses of aquatic organisms to environmental stress differs drastically between different species, and even between different clonal populations of the same species. Further work is required to fully understand the implications of the context-dependant plastic responses of this important aquatic keystone species, to the various anthropogenic changes that are occurring in aquatic ecosystems, across the globe. Furthermore, improving the understanding of the genetic basis of the responses of keystone species to environmental change will enable a more predictive understanding of the outcome of human impacts when they occur and may give insights on how we can better protect aquatic biodiversity.

14. Appendices

14.1 Appendix 1

Sequences have been edited to remove pGEM-T Easy Vector sequences and any parts of the sequence that contained bases that did not pass the confidence threshold. Raw sequences are available on request. Due to the 16s rRNA gene being highly conserved and our sequences representing only part of the 16s gene (after trimming), there are often multiple matches and one of them matched with two closely related symbionts. The best BLAST match is shown and if the distance tree of results suggests another bacterium is more closely related then this is also displayed. The sequences will be submitted to GenBank upon submission of chapter 11 to a peer-reviewed journal.

14.1.1 N116 aphid symbiont BLAST analysis

Trimmed 'N116' clone *Acyrthosiphon pisum* 16s rRNA gene sequences used in the blast analysis.

>ID: N2_2-T7

AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAGGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTGATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GagAAAGACTGACGCTGAGGTGCGAAAGCGTGGGgAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCTGTAAACGATGTcgATTTGgaGGTTGCGGTC
TTGAACTGTGgCGTCCGGAGCTAACGCG

- Candidatus Hamiltonella defensa strain AS3 chromosome
- Max score1581
- Total score 4745
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1

>ID: N4_1-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGGGAAGGTAGCTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGATTAGCTAG
TAGGAGAGGTAATGGCTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATTACTGGGCATAAAGGGCACGTAGGCGGTTTCTTA
AGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACTG
AGAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGA
AATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCcTGGA
CAGAGACTGACGCTGaGgTGCGAAAGCGTGGGTAGCAAACAG

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1430
- Total score 7124
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N51_2-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGGGAAGGTAGCTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGTCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGATTAGCTAG
TAGGAGAGGTAATGGCTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATTACTGGGCGTAAAGAGCACGTAGGCGGTTTCTTA
AGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACTG
AGAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGA
AATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGA
CAGAGACTGACGCTGAGGTGCGAAAGCGTGGgTAGCAAACAGGATTAGA
TACCcTGGTAGTCCACGCTGTAAACGATGTCGATTTGTAGGTTGTGGTTAT
AAACTGTGGCTTGCGGAGCAAACGCGTTAAATCGACCGCCTGGGgAGTAC
GGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGATGCCACGCGAAGAACCTTACCTACTCTT
GACATCCAGAGG

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1829

- Total score 9102
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N54_2-T7
AGAGTTTGATCCTGGCTCAGGTTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
CTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GAGAAAGACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCTGTAAnCGATGTCGATTTGGAGGTTGCGG
TCTTGAACTGTGGCGTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGA
GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAgCATGTGGTTTAATTCGATGCAACGCGAA

- Candidatus Hamiltonella defensa strain AS3 chromosome
- Max score1773
- Total score 5321
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1

>ID: N65_2-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGGGAAGGTAGCTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGATTAGCTAG
TAGGAGAGGTAATGGCTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGCACGTAGGCGGCTTCTT

AAGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACT
GAGAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTG
AAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGG
ACAGagACTGACGCTgaGGTGCGAAAGCGTGGgTAGCAAnCAGGATTAGAT
ACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGTAGGTTGTGGTTAT
AAACTGTGGCTTGCG

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1561
- Total score 7779
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N76_2-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCAAACACAT
GCAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGC
GAGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGG
GATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAA
AGTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATT
AGCTGGTAGGTAAGGTAAAGGCCTACCTAGGCGACGATCTCTAGCGGGT
CTGAGAGGATAGCCCGCCACACTGGAACTGAGAGACGGTCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC
AGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAG
CGAGGAGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCG
CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAG
GGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGGGCACGTAGGCGGT
TTCTTAAGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTG
AAACTGAGAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAG
CGGTGAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCC
CCTGGACAGAGACTGACGCTGAGGTGCGAAAGCGTGGgTAGCAAnCAGG
ATTAGATACCCTGGTAGTCCACGCTGTAAnCGATGTCGATTtGTAGGTTGT
GGTTATAAACTGTGGCTTGCGGAGCAAnCGCGTTAAATCGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAACTCAA

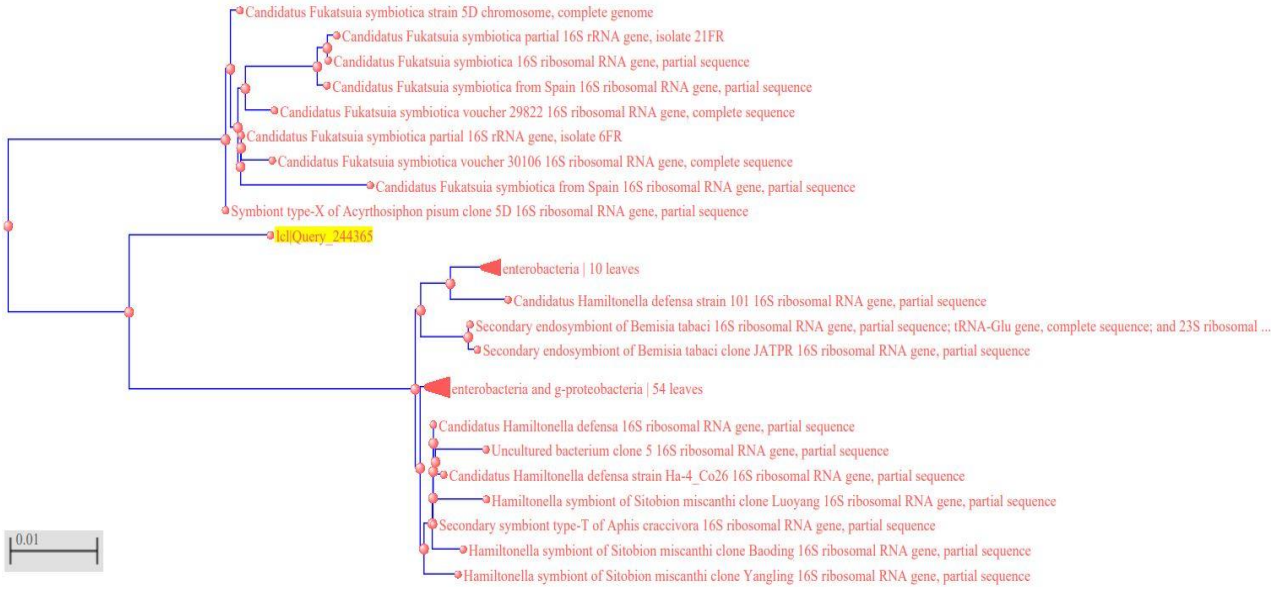
Best BLAST match is:

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1423
- Total score 7087
- Query cover 100%
- E value 0.0
- Indent 95%
- Accession no. CP021659.1

BUT

The distance tree of results suggests that this sequence is most closely related to *Hamiltonella defensa* but on a separate branch to the known *H. defensa* strains and is likely to be an unknown strain of *H. defensa*.

- Candidatus Hamiltonella defensa strain
- Max score 1421
- Total score 4263
- Query cover 100%
- E value 0.0
- Indent 95%
- Accession no. CP017610.1



>ID: N101_2-T7

AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACTGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
gaGAAAGACTGACGCTGAGGTGCGAAAGCGTGGGgAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGGT
CTTGAACTGTGGCGTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGgAG
TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG

CGGTGGagcATGTGGTTTAATTCGATGCAACGC

- Candidatus Hamiltonella defensa strain AS3 chromosome
- Max score 1777
- Total score 5332
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1

>**ID: N201_4-T7**
GGTTACCTTGTTACGACTTCACCCCAGTCATGGTTCACAAAGTGGTAAGC
GCCATCCCAAAGGTTAAGCTACCTACTTCTTTTGCAAAACATTCCCATGG
TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTC
TGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACT
CCAATCCGGACTACGACGTACTTTATGAGGTCCGCTCACCCTCGCAGGCT
CGCTTCTCTTTGTATACGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAG
GGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGC
AGTCTCTCTTGAGTTCCCACCTCTACGTGCTGGCAACAAAAGATAAGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGAC
AGCCATGCAGCACCTGTCTCAAAGCTCCCCGAAGGGCACGTCAACATCTC
TGTCGACTCCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATC
GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCG

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1085
- Total score 5419
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N203_4-T7
GGTTACCTTGTTAGGACTTCACCCCACTCATGAATCACAGACTGGTAAGC
GCCCTCCTTGCGGTTAAGCTACCTACTTGCTTTGCAACCCGATCCCATGGT
GAGACGGGAGGTGTGTACAAGGCCCGGGAACGTATTCACCGAAACATTC
TGATCTACGATTACTATACGATTCCGACTTCATGGAGTCAAGTGGCGGAC
TCCAATCCGGACTACCACCTAATTTCTGAGTTCGGCTTTCCCTCGCAGGTG
CGCATCCCTTTGTATACGCCATTGATCCACGTGTGTACCCCTACTCGGAA
CGGCCATGATGACTTGGCGACGTCCCCACCATCCTCCGGTTTAGCACCGG
TGGTCACCTTTGAGTTCCCGCCTCTACCCGCTGGCAACCAAGGACAAGGG
TTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACAAGCTGACGA
CGGCCATGCAGCACCTGTCTCACGGTTCCCGAAGGCACTTGCGTATCTCT
GCACAATTCCGTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCG
AATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTG
AGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTAG
CTCCGGACGCCACAGTTCAAGACCGCAACCTCCAAATCGACATCGTTTAC
AGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCA
CCTCAGCGTCAGTCTTTCTCCAGGGGGGCCGCCTTCGCCACCGGTATTCC

TCCAGATATCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTA
GAAAACTCTAGCGACCCAGTTTCAAATGCCATTCCCAAGATTGAGCTCGG
GGGATTTCACATCTGACTTAAATCCACCGCCTACGTGCCCTTTACGCCAG
TTAATTCCGATTAACGGCTCGCACCCTTCCGTATTACCGCGGCTTGCTGGC
ACGAGTTAGCGGTGCTTCTCCTGCAAGTAACGTCAAAGATAAAGTGCATT
CACATTTATCCCTTCTCCTCCTCGCTGAAAGTTCTTTATAAACCCGAAAGG
CCTTCTTC

- Candidatus Hamiltonella defensa strain AS3 chromosome
- Max score 1674
- Total score 5022
- Query cover 100%
- E value 0.0
- Indent 94%
- Accession no. CP017610.1

>ID: N204_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTC
CGAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCA
GCCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGC
GAGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCA
GAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGG
TGCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGA
GTTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAA
ACTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCG
GTGAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC
TGGAGAAAGACTGACGCTGAGGTGCGAAAGCGT

- Candidatus Hamiltonella defensa strain AS3 chromosome
- Max score 1408
- Total score 4224
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1

>**ID: N205_4-T7**
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGTGTGAGCGCAGTTTACTGAGTTCATGTCGGCGA
GCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGGAT
AACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAAGT
GGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTAGCT
GGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCTGA

GAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCC
ATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAG
GAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGTGGTGAGTT
AAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAACT
GGGTCGCTAGGGTTTTCTAGGGGGGGTAGAATTCCAGGTGTAGCGGTGA
AATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGA
GAAAGACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGGTC
TTGAACTGTGGCG

- Candidatus Hamiltonella defensa strain
- Max score 1546
- Total score 4629
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP022932.1, CP017610.1 (same species)

>ID: N206_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGGGAAGGTAGTTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGTTTAGCTAGT
AGGAGAGGTAATGGCTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG
CCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGA
GGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCG
AGCGTTAATCGGAATTACTGGGCGTAAAGAGCACGTAGGCGGTTTCTTAA
GTCAGACGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACTGA
GAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGAC
AGAGACTGACGCTGAGGTGCGAAAGCGTGGGTAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGTA

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1504
- Total score 7477
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N207_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATA

CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GTGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GAGAAAGACTGACGCTGAGGTGCGAAAGC

- Candidatus Hamiltonella defensa strain
- Max score 1399
- Total score 4197
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1, CP017606.1.

>ID: N208_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAGGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GAGAAAGACTGACGCTGAGGTGCGAAAGCG

- Candidatus Hamiltonella defensa strain
- Max score 1406
- Total score 4219
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1, CP017606.1.

>ID: N210_4-T7

GGTTACCTTGTTACGACTTCACCCCAGTCATGGTTCACAAAGTGGTAAGC
GCCATCCCAAAGGTTAAGCTACCTACTTCTTTTGCAAAACACTCCCATGG
TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTC
TGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACT
CCAATCCGGACTACGACGTACTTTATGAGGTCCGCTCACCCTCGCAGGCT
CGCTTCTCTTTGTATACGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAG
GGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGC
AGTCTCTCTTGAGTTCCCACCTCTACGTGCTGGCAACAAAAGATAAGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGAC
AGCCATGCAGCACCTGTCTCAAAGCTCCCCGAAGGGCACGTCAACATCTC
TGTCGACTCCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATC
GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTT
GAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTT
GCTCCGCAAGCCACAGTTTATAACCACAACCTACAAATCGACATCGTTTA
CAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCTCGCTTTCGC
ACCTCAGCGTCAGTCTCTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCC
TCCAGATATCTACGCATTTCACCGCTACACCTGGAAATTCTACCCCCCCTC
TACAAAACTCT

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1576
- Total score 7853
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N211_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTAGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGActgACGCTCAGGtGCGAAAGC

- Serratia symbiotica isolate Ap-1 16S ribosomal RNA gene, partial sequence
- Max score 1310
- Total score 1310

- Query cover 92%
- E value 0.0
- Indent 99%
- Accession no. , MG257483.1, BLAST also matched MF062650.1, KX900450.1 (same species)

>ID: N212_4-T7

GGTTACCTTGTTACGACTTCACCCCAGTCATGGTTCACAAAGTGGTAAGC
GCCATCCCAAAGGTTAAGCTACCTACTTCTTTTGCAAAACACTCCCATGG
TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTC
TGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACT
CCAATCCGGACTACGACGTACTTTATGAGGTCCGCTCACCCTCGCAGGCT
CGCTTCTCTTTGTATACGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAG
GGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGC
AGTCTCTCTTGAGTTCCCACCTCTACGTGCTGGCAACAAAAGATAAGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGAC
AGCCATGCAGCACCTGTCTCAAAGCTCCCCGAAGGGCACGTCAACATCTC
TGTCGACTCCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATC
GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTT
GAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTT
GCTCCGCAAGCCACAGTTTATAACCACAACCTACAAATCGACATCGTTTA
CAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCACGCTTTCGC
ACCTCAGCGTCagTCTCTGTCCAGGGGGG

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1439
- Total score 7192
- Query cover 99%
- E value 0.0
- Indent 100%
- Accession no. CP021659.1

>ID: N213_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GAGAAAGACTGACGCTGAGGTGCGAAAGC

- Candidatus Hamiltonella defensa strain
- Max score 1410
- Total score 4230
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1, CP017606.1.

>N214_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAACGGCATCGGGAAGGTAGCTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGATTAGCTAG
TAGGAGAGGTAATGGTTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGCACGTAGGCGGTTTCTTA
AGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACTG
AGAGTCTAGAGTTTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGA
AATGCGT

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1269
- Total score 6321
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N215_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAA

- Candidatus Hamiltonella defensa strain MI47 genome
- Max score 1315
- Total score 3933
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP022932.1

>ID: N217_4-T7
GGTTACCTTGTTACGACTTCACCCCAGTCATGAATCACAAAGTGGTAAGC
GCCTTCCTTTTAAAGGGTTAGGATACCTGCTTCTTTTGCAACCCACTCCCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGC
ATTCTGATCCACGATTACTAGCGATTCCGACTTCGTGGAGTCGAGTTGCA
GACTCCatTCCGGACTACGATTTACTTTATGAGGTTTGCTTGTCTTTGCAGA
TTTGCTTCTCTTTGTATAAACCATTGTAGCACGTGTGTAGCCCTGGTCGTA
AGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATAACCG
GCAGTCTCCTCTGAGTTCCCGGCCGAACCGCTGGCAACAGGGGATAAGG
GTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACG
ACAGCCATGCAGCACCTGTCTCACAGCTCCCGAAGGCACTTCTTTATTTC
TAAAGAATTCTGTGGATGTCAAGACCAGGTAAGGTTTTTCGCGTTGCATC
GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTT
GAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAATGCGTTA
GCTTCGGAAGTCACTTCTCTTGGAAACAACCTCCAAGTCGACATCGTTTA
CGGCATGGACTACCAGGGTATCTAATCCTGtTTGCTCCCCACGCtTTCGCG
CCTCAGTGTC

- Buchnera aphidicola str. JF99 (Acyrthosiphon pisum), complete genome
- Max score 1404
- Total score 1404
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP002302.1

>ID: N218_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAGA
GTGGGGGACCTTTGGGCCTCACGCCTTCGGATGAGCCCAGATGTGATTAG
CTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCTG
AGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCCCAATGGGCGAAAGCCTGATGCAGC
CATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGA
GGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTG
CGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAGT
TAAGCCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA

CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT

- Candidatus Hamiltonella defensa strain MI47 genome
- Max score 1243
- Total score 3716
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP022932.1

>ID: N219_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG
AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGATATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GAGAAAGACTGACGCTGAGG

- Candidatus Hamiltonella defensa strain
- Max score 1393
- Total score 4180
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1,CP017606.1.

>ID: N220_4-T7
GGTTACCTTGTTACGACTTCACCCCAGTCATGAATCACAAAGTGGTAAGC
GCCCTCCTTGCGGTTTAGCTACCTACTTCTTTTGCAACCCACTCCCATGGT
GTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCT
GATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTC
CAATCCGGACTACGACATACTTTCTGAGTTCCGCTTTCCCTCGCAGGTTCG
CATCCCTTTGTATACGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGG
GCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCA
GTCTCCTTTGAGTTCCCGCCTCTACGCGCTGGCAACAAAGGACAAGGGAT
GCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACA
GCCATGCAGCACCTGTCTCACGGTTCCCGAAGGCACTTGCGCATCTCTGC
ACAATTCCGTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAA
TTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAG

TTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTAGCT
CCGGACGCCACAGTTCAAGACCGCAACCTCCAAATCGACATCGTTTACAG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCT
CAGCGTCAGTCTTTCTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCA
GATATCTACGCATTTCACCGCTACACCT

- Candidatus Hamiltonella defensa strain
- Max score 1519
- Total score 4557
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP017610.1, CP017606.1, CP022932.1.

>ID: N221_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGGGAAGGTAGCTTGCTATCTTTGCCGGCGAGCG
GCGGACGGGTGAGTAAAGTCTGGGGATCTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATGATGTTACGCGACCAAAGCGGG
GGACCTCCGGGCCTCGCGCCATCAGATGAACCCAGATGGGATTAGCTAG
TAGGAGAGGTAATGGCTCCCCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATAACCAGCCACACTGGAACTGAGAGACGGTCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAATGAAGCAATGCAAAGAGTGTTGCTAATGGACGTTACTCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
GAGCGTTAATCGGAATTACTGGGCGTAAAGGGCACGTAGGCGGTTTCTTA
AGTCAGATGTGAAATCCCCGAGCTTCACTTGGGAACGGCATTTGAAACTG
AGAGTCTAGAGTTTTGTAGAGGGGGGTAGAAT

- Candidatus Fukatsuia symbiotica strain 5D chromosome, complete genome
- Max score 1236
- Total score 6154
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP021659.1

>ID: N222_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACACTGGTGGCAGGCCTAACACATG
CAAGTCGAGCGGCATCGAGTGAGCGCAGTTTACTGAGTTCATGTCGGCG
AGCGGCGGACGGGTGAGTAAAGTCTGGGAATCTGGCCGAAGGAGGGGG
ATAACTGCTGGAAACGGCAGCTAATACCGCATGAAGTCGCGAGACCAAA
GTGGGGGACCTTCGGGCCTCACGCCTTCGGATGAGCCCAGATGAGATTA
GCTGGTAGGTAAGGTAAAGGCTTACCTAGGCGACGATCTCTAGCGGGTCT
GAGAGGATAGCCCGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CCATGCCACGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
AGGAGGAAGCGATAAATGCGAATACCATTTATTTTTGACGTTACTCGCAG

AAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGT
GCGAGCGTTAATCGGAATAACTGGGCGTAAAGGGCATGTAGGCGGTGAG
TTAAGTCAGATGTGAAATCCCCGAGCTCAACTTGGGAATGGCATTTGAAA
CTGGGTCGCTAGAGTTTTCTAGAGGGGGGTAGAATTCCAGGTGT

- Candidatus Hamiltonella defensa strain MI47 genome
- Max score 1260
- Total score 3766
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP022932.1

14.1.2 Quad aphid symbiont BLAST analysis

Trimmed ‘Quad’ clone *Acyrthosiphon pisum* 16s rRNA gene sequences used in the blast analysis.

>ID: Q100_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTAGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGG

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica isolate Ap-1 16S ribosomal RNA gene, partial sequence
- Max score 1125
- Total score 1125
- Query cover 91%
- E value 0.0
- Indent 99%
- Accession no. MG257483.1, BLAST also matches MF062650.1, KX900450.1 (same species)

>ID: Q101_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA

GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGCCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAGGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCTGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTC

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica gene for 16S rRNA, partial sequence, host: Acyrthosiphon pisum line P136
- Max score 1188
- Total score 1188
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AB522706.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species)

>ID: Q102_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTT

- Candidatus Serratia symbiotica isolate KoGrPit clone 9d3 16S ribosomal RNA gene, partial sequence
- Max score 776
- Total score 776
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. GU592763.1, BLAST also matches MF062650.1, KX900450.1, GU592768.1, GU592756.1 (same species).

>ID: Q103_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG

CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGT

Distance tree of results shows that the most closely related species is:

- Candidatus Serratia symbiotica 16S ribosomal RNA gene, partial sequence; tRNA-Glu gene, complete sequence; and 23S ribosomal RNA gene, partial sequence
- Max score 1158
- Total score 1158
- Query cover 95%
- E value 0.0
- Indent 100%
- Accession no. AY296732.1, BLAST also matches M27040.1, MF062650.1, KX900450.1. (same species).

>ID: Q104_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAGGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTAGGAACGGCATTTGAGACTGGC
AA

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica isolate
- Max score 1081
- Total score 1081
- Query cover 91%

- E value 0.0
- Indent 99%
- Accession no. MG257483.1, BLAST also matches MF062650.1, KX900450.1 (same species).

>ID: Q105_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCG

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica isolate A1 16S ribosomal RNA gene, partial sequence
- Max score 1275
- Total score 1275
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. KX900450.1, BLAST also matches MF062650.1 (same species).

>ID: Q106_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCgngGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
C

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica isolate A1 16S ribosomal RNA gene, partial sequence

- Max score 990
- Total score 990
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. KX900450.1, BLAST also matches MF062650.1 (same species).

>ID: Q107_4-T7

GGTTACCTTGTTACGACTTCACCCCAGTCATGAATCACAAAGTGGTAAGC
GCCTTCCTTTTAAAGGGTTAGGATACCTGCTTCTTTTGCAACCCACTCCCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGC
ATTCTGATCCACGATTACTAGCGATTCCGACTTCGTGGAGTCGAGTTGCA
GACTCCAGTCCGGACTACGATTTACTTTATGAGGTTTGCTTGTCTTTGCAG
ATTTGCTTCTCTTTGTATAAACCATTGTAGCACGTGTGTAGCCCTGGTCGT
AAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATAACC
GGCAGTCTCCTCTGAGTTCCCGGCCGAACCGCTGGCAACAGGGGATAAG
GGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGAC
GACAGCCATGCAGCACCTGTCTCACAGCTCCCGAAGGCACTTCTTTATTT
CTAAAGAATTCTGTGGATGTCAAGACCAGGTAAGGTTTTTCGCGTTGCAT
CGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATT
TGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAATGCGTT
AGCTTCGGAAGTCACTTCTCTTGGAAACAACCTCCAAGTCGACATCGTTT
ACGGCATGGACCACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCG
CGCCTCAGTGTCAGTTTTT

BLAST matched with 6 strains of Buchnera aphidicola all with the same results

- Buchnera aphidicola str.
- Max score 1415
- Total score 1415
- Query cover 99%
- E value 0.0
- Indent 99%
- Accession no. CP002302.1, CP002301.1, CP002300.1, CP001161.1, CP001158.1, BA000003.2.

>ID: Q108-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA

GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGG

The distance tree of results suggests that the most closely related is:

- Candidatus Serratia symbiotica 16S ribosomal RNA gene, partial sequence; tRNA-Glu gene, complete sequence; and 23S ribosomal RNA gene, partial sequence
- Max score 1112
- Total score 1112
- Query cover 96%
- E value 0.0
- Indent 100%
- Accession no. AY296732.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species).

>ID: Q113_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCAGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGG

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica
- Max score 1238
- Total score 1238
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. MF062650.1, KX900450.1, KP866561.1 (same species).

>ID: Q114_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG

ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGT

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica isolate
- Max score 1068
- Total score 1068
- Query cover 95%
- E value 0.0
- Indent 99%
- Accession no. KX900450.1, BLAST also matches MF062650.1, KX900450.1 (same species).

>ID: Q115_4-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTG
AGGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCCTGGGGGAGTA
CG

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica A1 16S ribosomal RNA gene, partial sequence
- Max score 1616
- Total score 1616
- Query cover 100%
- E value 0.0

- Indent 99%
- Accession no. KX900450.1, MF062650.1 (same species).

>ID: Q116_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCCTT
G

The distance tree of results suggests that the most closely related is:

- Candidatus Serratia symbiotica 16S ribosomal RNA gene, partial sequence; tRNA-Glu gene, complete sequence; and 23S ribosomal RNA gene, partial sequence
- Max score 1491
- Total score 1491
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AY296732.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species).

>ID: Q117_4-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCCTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG

CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGA

The distance tree of results suggests that the most closely related is:

- Candidatus Serratia symbiotica 16S ribosomal RNA gene, partial sequence; tRNA-Glu gene, complete sequence; and 23S ribosomal RNA gene, partial sequence
- Max score 1258
- Total score 1258
- Query cover 96%
- E value 0.0
- Indent 99%
- Accession no. AY296732.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species).

>ID: Q2_1-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCCGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCC

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica A1 16S ribosomal RNA gene, partial sequence
- Max score 1105
- Total score 1105
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. KX900450.1, BLAST also matches MF062650.1 (same species).

>ID: Q4_1-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG

ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAAAGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGC

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica A1 16S ribosomal RNA gene, partial sequence
- Max score 885
- Total score 885
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. KX900450.1, BLAST also matches MF062650.1 (same species).

>ID: Q6_1-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAAGCCTAACACATG
CAAGTCGAGCGGCAGCGAGAAGAGAGCTTGCTCTCTTTGTCGGCAAGCG
GCAAACGGGTGAGTAATATCTGGGGATCTACCCAAAAGAGGGGGATAAC
TACTAGAAATGGTAGCTAATACCGCATAATGTTGAAAAACCAAAGTGGG
GGACCTTTTGGCCTCATGCTTTTGGATGAACCCAGACGAGATTAGCTTGT
TGGTAGAGTAATAGCCTACCAAGGCAACGATCTCTAGCTGGTCTGAGAG
GATAACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCTATG
CCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAAAAAATAAAACTAATAATTTTATTTCGTGACGTTACCCGCAGAAG
GAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCAGAATTACTGGGCGTAAAGAGCGCGTAGGTGGTTTTTTAA
GTCAGGTGTGAAATCCCTAGGCTCAACCTAGGAACTGCATTTGAAACTGG
AAAACTAGAGTTTCGTAGAGGGAGGTAGAATTCTAGGTGTAGCGGTGAA
ATGCGTAGATATCTGGAGGAATACCCGTGGCGAAAGCGGCCTCCTAAAC
GAAAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCATGCCGTAAACGATGTCGACTTGGAGGTTGTTTCCA
AGAGAAGTGACTTCCGAAGCTAACGCATTAAGTCGACCGCCTGGGGGAG
TACGGCCGCAAGGCTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
C

BLAST matches with 5 strains of Buchnera aphidicola, all with the same results.

- Buchnera aphidicola str.
- Max score 1711
- Total score 1711
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP002301.1, CP002300.1, CP001161.1, CP001158.1,

BA000003.2.

>ID: Q7_1-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAAGCCTAACACATG
CAAGTCGAGCGGCAGCGAGAAGAGAGCTTGCTCTCTTTGTCGGCAAGCG
GCAAACGGGTGAGTAATATCTGGGGATCTACCCAAAAGAGGGGGATAAC
TACTAGAAATGGTAGCTAATACCGCATAATGTTGAAAAACCAAAGTGGG
GGACCTTTTGGCCTCATGCTTTTGGATGAACCCAGACGAGATTAGCTTGT
TGGTAGAGTAATAGCCTACCAAGGCAACGATCTCTAGCTGGTCTCAGAG
GATAACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCTATG
CCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAAAAAATAAAACTAATAATTTTATTTCGTGACGTTACCCGCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCAGAATTACTGGGCGTAAAGAGCGCGTAGGTGGTTTTTTAA
GTCAGGTGTGAAATCCCTAGGCTCAACCTAGGAACTGCACTTGAAACTGG
AAAACTAGAGTTTCGTAGAGGGAGGTAGAATTCTAGGTGTAGCGGTGAA
ATGCGTAGATATCTGGAGGAATACCCGTGGCGAAAGCGGCCTCCTAAAC
GAAAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAG

Matched with 6 strains of Buchnera aphidicola all with the same results

- Buchnera aphidicola str.
- Max score 1424
- Total score 1424
- Query cover 100%
- E value 0.0
- Indent 99%
- Accession no. CP002301.1, CP002300.1, CP001161.1, CP001158.1, BA000003.2, M27039.1.

>ID: Q8_1-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCGTAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGCAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCAGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTG

AGGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCCTGGGGAGTAC
GGCCGcAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGATGCAACGCG

The distance tree of results suggests that the most closely related is:

- Candidatus Serratia symbiotica 16S ribosomal RNA (rrs) gene, partial sequence
- Max score 1703
- Total score 1703
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AF293617.1, BLAST also matches MF062650.1, MF062649.1, KX900450.1, KT175992.1, M27040.1 (same species).

>ID: Q9_1-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCGAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAGCTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTG
AGGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCC

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica gene for 16S rRNA, partial sequence, host: Acyrthosiphon pisum line P136
- Max score 1570
- Total score 1570
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AB522706.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species).

>ID: Q10_1-T7

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACATCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTGGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTG
AGGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCCTGGGGAGTAC
GGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC

The distance tree of results suggests that the most closely related is:

- Secondary symbiont of Acyrthosiphon pisum gene for 16S rRNA
- Max score 1727
- Total score 1727
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AB033777.1, BLAST also matches MF062650.1, MF062649.1, KX900450.1, KT175992.1, M27040.1 (same species).

>ID: Q12_1-T7
AGAGTTTGATCCTGGCTCAGATTgaACGCTGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGC
GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTA
GTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGG
ACCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTGGGATTAGCTGGTAG
GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA
TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
GCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTACAGCGAGAAGA
AAGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAA
GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGT
CAGATGTGAAATCCCCGCGCTCAACGTAGGAACGGCATTTGAGACTGGC
AAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGCGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTG
AGGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCCTGGGGAGTAC

GGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGaGCATGTGGTTTAATTCGATGCAACGCGA
The distance tree of results suggests that the most closely related is:

- Secondary symbiont of Acyrthosiphon pisum gene for 16S rRNA
- Max score 1727
- Total score 1727
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AB033777.1, BLAST also matches MF062650.1, MF062649.1, KX900450.1, KT175992.1, M27040.1.

>ID: Q14_1-T7
AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCGGCCTAACACATGC
AAGTCGAGCGGTAGCACAAGAGAGCTTGCTCTCTGGGTGACGAGCGGCG
GACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGCGGGGGATAACTAG
TGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGA
CCTTCGGGCCTCACGCCATCAGATGTGCCCAGGTAGGATTAGCTGGTAGG
TGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA
GCAGCGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG
CGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACCTTCAGCGAGGAGAA
AGGGTAATGTGTTAATAAGACATTGCATTGACGTTACTCGCAGAAGAAG
CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGC
GTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTC
AGATGTGAAATCCCCGCGCTCAACGGGGGAACGGCATTTGAGACTGGCA
AGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAAT
GCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAA
AGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGCGCCCTTGA
GGGGTGGCTTCCGTAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACG
GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGT

The distance tree of results suggests that the most closely related is:

- Serratia symbiotica gene for 16S rRNA, partial sequence, host: Acyrthosiphon pisum line P136
- Max score 1685
- Total score 1685
- Query cover 97%
- E value 0.0
- Indent 99%
- Accession no. AB522706.1, BLAST also matches M27040.1, MF062650.1, KX900450.1 (same species).

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