GENETICS OF FORAGING BEHAVIOR OF THE PREDATORY MITE, *PHYTOSEIULUS PERSIMILIS*

by

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ABSTRACT

Phytoseiulus persimilis (Acari: Phytoseiidae) is a specialist predator on tetranychid mites, especially on the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). The foraging environment of the predatory mites consists of prey colonies distributed in patches within and among plants. Quantitative genetic studies have shown genetic variation in, and phenotypic correlations among, several foraging behaviors within populations of the predatory mite, *P. persimilis*. The correlations between patch location, patch residence, consumption and oviposition imply possible fitness trade-offs. We used molecular techniques to investigate genetic variation underlying the foraging behaviors. However, these genetic studies require a sufficiently large amount of DNA which was a limiting factor in our studies. Therefore, we developed a method for obtaining DNA from a single mite by using a chelex extraction followed by whole genome amplification. Whole genome amplification from a single mite provided us with a large quantity of high-quality DNA. We obtained more than a ten thousand-fold amplified DNA from a single mite using 0.01ng as template DNA. Sequence polymorphisms of *P. persimilis* were analyzed for nuclear DNA Inter Transcribed Spacers (ITS1 & ITS2) and for a mitochondrial12S rRNA. The sequence comparisons among individuals identified a number of polymorphisms in the 12S sequences.

The foraging gene (*for*) associated with rover-sitter behavioral strategies of *Drosophila* is known to have role in feeding behaviors of honeybee and other arthropods. We surmised that the same or a similar gene may be present in *P. persimilis*. Among the foraging behavior(s) exhibited by this predatory mite, we were particularly interested in resource/prey-dependent dispersal behavior. We isolated a partial sequence that is presumed to be the orthologue of the foraging (*for*) gene. We named the putative foraging gene as *Ppfor* (for *Phytoseiulus persimilis* foraging gene). We used a fragment of *Ppfor* gene as a molecular marker between populations and among individuals and, further, to help understand behavioral phenotypes.

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INTRODUCTION

Phytoseiulus persimilis Athias-Henriot is a specialist predator on the twospotted spider mite, *Tetranychus urticae* Koch. This predatory mite was introduced into Netherlands from Chile in 1958, and subsequently was shipped to other parts of the world, including California and Florida from Germany. Adults are extremely small (approximately 0.5 mm), and orange to bright reddish orange. Eggs are oval and approximately twice as large as the pest mite eggs. Laing (1968) determined that *P. persimilis* would develop from egg to adult in an average of 7.45 days; this is approximately half the time required for development of the twospotted spider mites under similar conditions of temperature and relative humidity. *P. persimilis* is a fast moving predatory mite which searches leaf surfaces for webbing of pest mites. Once it encounters webbing it will intensify its search for eggs, larvae and adults of twospotted spider mites (Sabelis, 1981; Maeda *et al*, 1999; Mayland *et al*., 2000). In the webbing of its prey the female *P. persimilis* lays its eggs within the strands of silk, where her young will hatch and continue to feed. A well-fed female predator will lay about 40-50 eggs in her lifetime. Eggs are laid in close proximity to the food source. Therefore, the webbing produced by twospotted spider mites aids the searching predator in finding its prey.

The twospotted spider mite, *T. urticae,* is a serious pest on over 30 economically important crops, including corn, cotton, cucumbers, peanuts, sorghum, beans, melons, strawberries and a variety of greenhouse ornamentals. Foliar damage occurs because mites remove chlorophyll, water and nutrients from leaf cells. Spider mites deposit eggs close to where they feed and most offspring do not move very far from where they hatch; thus feeding is usually localized. Predatory mites have been used successfully to control spider mites in greenhouses, in

orchards, in vineyards and on strawberries.

Attraction of arthropod predators and parasitoids to volatile chemicals produced by plants attacked by herbivores has been demonstrated in the laboratory (e.g., Dicke *et al*., 1990 a, b). In field conditions, attraction to herbivore-induced plant chemicals may benefit a natural enemy searching for prey by increasing its search efficiency (Sabelis *et al*., 2001). Previous studies on attraction of *P. persimilis* to volatiles produced by spider mite-infested bean plants suggested considerable phenotypic variation in predator responsiveness. Van Baalaen and Sabelis (1995) proposed the probability of two different predator strategies: 'Killer' are the predators which remain in a patch as long as there are prey left; and 'Milker' are the predators which disperse at a constant rate from a patch. This variation may be attributable to the physiological state of the predator (Sabelis & Vande Baan, 1983; Dicke *et al.,* 1990 b; Zhang and Sanderson, 1993). However, genetic variation in some components of the search behavior has been suggested in predatory arthropods and parasitoids (Hopper *et al.,* 1993; Hoy, 1990).

In tritrophic systems, there is evidence for a genetic component in the response of parasitoid wasps to plant chemicals in terms of host acceptance (Mollema, 1991; Powell and Wright, 1992) and attraction to infested plants (Prevost and Lewis, 1990). Margolies *et al.* (1997) examined intrapopulation genetic variation in predator response to herbivore-induced volatiles in a tritrophic system consisting of bean plants (*Phaseolus lunatus* L.), the twospotted spider mite (*T. urticae*) and a predatory mite (*P. persimilis*). The selection response they observed is evidence of additive genetic variation in predator response to twospotted spider mite-induced plant volatiles; that is, genetic variation that can lead to evolution of the trait. Genetic diversity in natural enemy response to herbivore-induced plant volatiles may be a consequence of variability in the plant signal (Lewis *et al*., 1990; Vet and Dicke; 1992). Dicke *et al.* (1990a,

b); Lewis *et al.* (1990) and Vet and Dicke (1992) suggested that predator-prey dynamics could be affected by the genetic variation underlying olfactory responses. Subsequently, Jia *et al.* (2002) quantified genetic variation in, and phenotypic correlations among, several foraging behaviors of *P. persimilis*.

To effectively suppress the explosive growth of prey populations, phytoseiid mites must show a high numerical response, unless it is feasible to rear and release them in large numbers. Further, the predator response to spider mite-induced plant volatiles is likely to be important in the ability of a predator to reduce a prey population and, hence, to enhance their success as biological control agents. However, reproductive success depends to a major extent on the rate of prey consumption which, in turn, is link to prey-finding behavior. Thus, predators that search and select prey so as to maximize their contribution of progeny to the next generations are favored compared to those that have poor searching ability. Therefore it can be assumed that the behavioral responses elicited by cues from their prey have evolved because they led the predator to profitable prey species, i.e., profitable in terms of the predator's reproduction. By this reasoning, foraging traits like response to plant volatiles, prey consumption, and dispersal are significant in estimating the efficiency of the predator. Previous studies showed that response of predators to prey-induced plant volatiles are heritable.

for Gene, PKG and Food-Related Behavior

Sokolowski (1980) discovered that in nature larvae of the fruitfly, *Drosophila melanogaster*, exhibit one of two foraging strategies: ''Rover'' larvae travel long distances while feeding and often leave a food source in search of another. Conversely, ''sitter'' larvae travel shorter distances and remain on a food source once it is encountered. These behavioral differences reflect foraging and not general locomotion since rovers and sitters move similar

distances in the absence of food. Differences in rover and sitter behavior are attributable to a single major gene called *foraging (for)* that is located on the 2nd chromosome at polytene position 24A3–5 (de Belle *et al.*, 1989). The rover allele (for^R) is dominant to the sitter allele (for^s) (de Belle and Sokolowski, 1987). Both rovers and sitters are found in natural populations such that phenotypic variation is typically 70 % rover (for^R homozygotes and heterozygotes) and 30% sitter (for^s homozygotes) (Sokolowski, 1980; Sokolowski *et al.*, 1997). Sokolowski *et al.* (1997) provide evidence for the evolution of the rover/sitter behavioral polymorphism via density-dependent selection. Rovers are favored in high density environments and sitters are favored in low density environments.

Osborne *et al.* (1997) discovered that *for* is the gene encoding one of two cyclic GMP-dependent protein kinase (PKG) genes in *D. melanogaster* (*dg2*) that was previously described by Kalderon and Rubin (1989). The *for* is a large gene (approximately 40 kb of genomic DNA) and is alternatively spliced into three major transcripts (*for* T1, T2, & T3). A cGMP-binding domain and a kinase domain are common to all transcripts of *for* such that the 59 regions make the transcripts unique (Kalderon and Rubin, 1989; Osborne *et al.*, 1997). The T1 and T3 transcripts contain dimerization and regulatory domains which are absent in T2. Rovers typically have higher transcript abundance and PKG activity levels relative to sitters (Osborne *et al.*, 1997). Using transgenic flies, sitters can be turned into rovers by providing more PKG (Osborne *et al.*, 1997).

Ben-Shahar *et al.* (2002; 2003) demonstrated the role of PKG in the behavioral transition of honey bee (*Apis mellifera*) from nurse to forager. Nurse bees, which distribute food within the hive, have lower PKG activity levels and lower abundance of *Amfor* RNA (the honey bee orthologue of *for*). This is similar to sitter fruitflies that forage close to home. In contrast,

forager bees, which leave the hive in search of food, have higher PKG activity levels and higher RNA abundance much like rover flies that forage by moving from a patch to another patch of food. However, the differences in RNA abundance and PKG activity leading to changes in division of labour are not age-dependent (Ben-Shahar *et al.*, 2002). The causal relationship between PKG and behavioral task was demonstrated by feeding young bees with 8-Br-cGMP, an activator of PKG, which resulted in transition of nurse bees to foragers (Ben-Shahar *et al.*, 2002).

The PKG molecule encoded by the gene *elg-4* has also been shown to influence food-related behaviors in the nematode worm, *C. elegans*. Nematode locomotion is categorized into two types: roaming and dwelling. Roaming is defined by long distances of uninterrupted locomotion and dwelling involves short distances and frequent stops. Using *egl-4* knock outs, Fujiwara *et al.* (2002) showed that decreasing PKG causes an increase in roaming behavior on food. The roamer and dweller phenotypes are reminiscent of rover and sitter fruitflies and nurse and forager honey bees. However, in *C. elegans* mutational analysis suggests that less PKG causes more roaming than dwelling. Mutants in *egl-4* which have reduced PKG expression show defects in the long-term regulation of olfactory behaviors. These mutational analyses in *C. elegans* have identified new functions for PKG (Fujiwara *et al.,* 2002).

There also exists a natural polymorphism in the feeding behavior of C. *elegans*. Some individuals form aggregations while feeding (‘‘social’’) and others remain solitary (de Bono and Bargmann, 1998). Moreover, aggregating strains move slowly when feeding (like sitter flies), whereas strains that are solitary move faster (like rover flies). As with fruitflies, this behavior is dependent on the presence of food. These naturally occurring behavioral differences are attributed to variation in the gene *npr-1,* which encodes a G protein- coupled receptor similar to the neuropeptide Y (NPY) receptors found in mammals. Natural aggregating strains differ from

solitary strains by only a single amino acid at position 215 of NPR-1. Aggregating strains have a phenylalanine (NPR-1 215F) and solitary strains have a valine (NPR-1 215V). Insertion of an NPR-1 215V transgene into aggregating strains causes them to behave like solitary strains. Recent evidence suggests that solitary feeding is a result of the inhibition of aggregate feeding (Coates and de Bono, 2002). Expressed in neurons, the solitary NPR-1 215V isoform is thought to antagonize a cGMP-gated ion channel encoded by the genes *tax-2* and *tax-4.* Like mammalian NPY, therefore, nematode NPR is involved in the regulation of food related behaviors via the suppression of various neurons (Cowley *et al.*, 2001). Food-related behaviors in *C. elegans* involve aggregate *vs.* solitary feeding and roaming *vs.* dwelling. The former is regulated by a neuropeptide-Y-like receptor and the latter is influenced by PKG (Fujiwara *et al.*, 2002). Hence the regulation of foraging behavior in fruitflies, honey bees and nematodes revolve around the PKG.

Molecular Markers and Whole Genome Amplification

Insect geneticists have always had problems in maintaining the quality and quantity of the original template DNA. Genetic applications require large amounts of template for testing, yet typically the yield of DNA from individual insects has been small. With Whole Genome Amplification (WGA) it should be possible to extend indefinitely the number of markers that can be analyzed in terms of map-based positional identification of genes (Dean *et al*., 2001). WGA amplified DNA is useful for several methods of genetic analysis such as single nucleotide polymorphisms, nuclear sequencing, chromosomal mapping, etc. Dean *et al.* (2001) reported the production of 20-30 g of amplified DNA by adopting WGA. Gorrochotegui and Black (2003) demonstrated that WGA can work with as little as 14ng of original template of *Aedes aegypti*.

They also showed that genetic polymorphisms at individual loci for both original genomic DNA and WGA amplified DNA.

The patterns of evolution of animal mitochondrial DNA (mtDNA) and the use of it as a molecular marker of intraspecific variation are well understood (Avise, 1994; Simon *et al*., 1994). Nuclear ribosomal DNA (rDNA) has proven very useful in inferring species phylogenies at various evolutionary scales (Hilis and Dixon, 1991). Intra-individual heterogeneity of ITS sequences has been reported in two species of mosquitoes (Wesson *et al*., 1993), in black flies (Tang *et al*., 1996) and in beetles (Vogler and DeSalle, 1994). They suggested that this variability resulted from the fact that the species were subdivided into races, ecotypes or subspecies that had distinct histories but between which a certain amount of secondary exchange occurred and fulfilled the mixing of differentiated ITS sequences, delaying or preventing homogenization. Navajas *et al.* (1998) stated that inferring historical patterns of gene flow in species could be easy by studying both mtDNA and rDNA variation.

We are interested in using molecular techniques to investigate fitness consequences of foraging behavior(s) and how behavioral variation is maintained in different populations by looking at the polymorphisms. The purpose of this current study is to explore the genetic mechanisms behind the foraging behavioral polymorphism in predatory mite, *P. persimilis.* The objectives of my research were:

OBJECTIVES

1. To develop molecular markers for *Phytoseiulus persimilis*
   i. Whole genome amplification of DNA of individual mite, *P. persimilis*
   ii. Develop polymorphic molecular markers using nuclear ITS , mitochondrial 12 S sequences
2. To test whether the foraging gene (*for*) is involved in the foraging (dispersal) behavior of predatory mite, *P. persimilis*
   i. Check whether *for* gene sequence is present in *P. persimilis*
   ii. Clone the *for* gene in *P. persimilis* using degenerate primers
   iii. Explore the role of cGMP-dependant protein kinase (PKG) in foraging (dispersal) behavior of predatory mites
   iv. Develop a molecular marker on a part of the foraging gene for examining different geographical populations of *P. persimilis*

REFERENCES CITED

Avise, J. C. (1994). *Molecular markers, Natural History and Evolution*. Chapman and Hall, New York

Ben-Shahar, Y., Robichon, A., Sokolowski, M. B., and Robinson, G. E. (2002). Influence of gene action across different time scales on behavior. Science 296:741-744

Ben-Shahar, Y., Leung, H. T., Pak, W. L., Sokolowski, M. B., and Robinson, G. E. (2003). cGMP-dependant changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. J. Exp. Biol. 206:2507-2515

Coates, J. C., and de Bono, M. (2002). Antagonistic pathways in neurons exposed to body fluid regulate social feeding in *Caenorhabditis elegans.* Nature 419:925-929

Dean, F. B., Hosono, S., Fang, L., Xiaohong Wu, A., Faruqi, F., Bray-Ward, P., Sun, Z., Zong, Q., Du, Y., Driscoll, M., Song, Kingsmore, S. F., Egholm, M., and Lasken, R. S. (2002). Comprehensive human genome amplification using multiple displacement amplification. Proc. Natl. Acad. Sci. U.S.A 99:5261-5266

de Belle, J. S., and Sokolowski, M. B. (1987). Heredity of rover/sitter: Alternative foraging strategies of *Drosophila melanogaster* larvae. Heredity 59:73-83

de Belle, J. S., Hilliker, A. J., and Sokolowski, M. B. (1989). Genetic localization of foraging (*for*): A major gene for larval behavior in *Drosophila melanogaster*. Genetics 123:157-163

de Bono, M., and Bargmann, C. I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans.* Cell 94:679-689

Dicke, M., Van Beek,T. A., Posthumus, M. A., Ben Dom, N., Van Bokhoven, H., and de Groot, A. E (1990a). Isolation and identification of volatile kairamone that affect acarine predatory prey

interactions: Involvement of host plant in its production. J. Chem. Ecol. 16:381-396

Dicke, M., Sabelis, M. W., Takabayashi, J., Bruin, J., and Posthumus, M. A. (1990b). Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. J. Chem. Ecol. 16:3091-31118

Fujiwara, M., Sengupta, P., and McIntire, S. L. (2002). Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4-cGMP-dependant protein kinase. Neuron 36:1091-1102

Gorrochotegui -Escalante, N., and Black, W. C. (2003). Amplifying whole insect genomes with multiple displacement amplification. Insect Mol. Biol. 12:195-200

Gurnett, A., Liberator, P. A., Dulski, P., Salowe, S. P., Donald, R. G. K., Anderson, J. W., Wiltsie, J., Diaz-Saldana, C. A., Harris, G., Chang, B., Darkin-Rattray, S. J., Nare, B., Crumley,T., Blum, P., Misura, A., Tamas, T., Sardana, M., Yuan, J., Biftu, T., and Schmatz, D. (2002). Purification and molecular characterization of cGMP- dependent protein kinase from Apicomplexan parasites. A novel chemotherapeutic target. J. Biol. Chem. 277:15913– 15922

Hillis, D. M., and Dixon, M. T. (1991). Ribosomal DNA: molecular evolution and phylogenetic interference. Q. Rev. Biol. 66:411-453

Hopper, K. R., Roush, R. T., and Powell, W. (1993). Management of genetics of biological control introductions. Annu. Rev. Entomol. 38:27-51

Hoy, M. A. (1990). Genetic improvement of arthropod natural enemies: Becoming a conventional tactic? In Baker, R. R., and Dunn, P. E. (eds), *New Directions in* Biological Control, Alan R. Liss, New York:405-417

Jia, F., Margolies. D. C., Boyer, J. C., and Charlton, R. E. (2002). Genetic variation in foraging traits among inbred lines of a predatory mite. Heredity 89:371-379

Kalderon, D., and Rubin, G. M. (1989). cGMP-dependant protein kinase genes in *Drosophila*. J. Biol. Chem. 264: 10734-10748

Laing, J. E. (1968). Life history and life table of *Phytoseiulus persimilis* Athias-Henriot. Acarologia 10:578-88

Lewis, W. J., Vet, L. E. M., Temlinson, J. H., van Lenteren, J. C., and Papaj, D. R. (1990). Variations in parasitoid foraging behavior: Essential element of a sound biological control theory. Environ. Entomol. 19:1183-1193

Maeda, T., Takabayashi, J., Yano, S., and Takafuji, A. (1999). Response of the predatory mite, *Amblyseius womersleyi* (Acari: phytoseiidae), toward herbivore-induced plant volatiles: variation in response between two local populations. App. Entomol. Zoo. 34: 449-454

Margolies, D. C., Sabelis, M. W., and Boyer, Jr. J. E. (1997). Response of a Phytoseiid Predator to Herbivore Induced Plant Volatiles: Selection on Attraction and Effect on Prey Exploitation. J. Insect. Behav. 10:695-709

Mayland, H., Margolies, D.C., and Charlton, R.E. (2000). Multiple prey related cues influence residence time of an acarine predator, *Phytoseiulus persimilis,* on prey host plants. Entom. Exp. Apl. 96:245-252

Mollema, C. (1991). Heritability estimates of host selection behavior by the *Drosophila* parasitoid *Asobara tabida.* Neth. J. Zool. 41:174-183

Navajas, M., Lagnel, J., Gutierrez, J., and Boursot, P. (1998). Species- wide homogeneity of nuclear ribosomal *ITS2* sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heridity 80:742-752

Osborne, K, A., Robichon, A., Burgess, E., Butland, S., Shaw, R. A., Coutland, A., Pereria, H. S., Greenspan, R. J., and Sokolowski, M. B, (1997). Natural behavioral polymorphism due to

cGMP-dependant protein kinase of *Drosophila.* Science 277:834-836

Powell, W., and Wright, A. F. (1992). The influence of host food plants on host recognition by four aphidiine parasitoids (Hymenoptera: Braconidae) Bull. Entomol. Res. 81:449-453

Prevost, G., and Lewis, W. J. (1990). Heritable differences in the response of the braconid wasp *Microplitis croceipes* to volatile allelochemicals. J. Insect. Behav. 3:277-287

Sabelis, M.W. (1981). Biological control of two spotted spider mites using phytoseiid predators. I. Agric. Res. Report 910. Pudoc, Wageningen, the Netherlands

Sabelis, M. W., and Van de Baan, H. E. (1983). Location of distant spider mite colonies by phytoseiid predators: Demonstration of specific kairamones emitted by *Tetranychus urticae* and *Panonychus ulmi.* Entomol. Exp. Appl. 33:303-314

Sabelis, M. W., Janssen, A and Kant, R. M. (1983). The Enemy of My Enemy is My Ally. Science 291:2104-2105

Sokolowski, M. B., (1980). Foraging strategies of *Drosophila melanogaster*: A chromosomal analysis. Behav. Gen. 10:291-302

Sokolowski, M. B., Pereira, H. S., and Hughes, K. (1997). Evolution of foraging behavior in *Drosophila* by density- dependent selection. Proc. Natl. Sci. U.S.A. 94:7373-7377

Tang, J., Toe, L., Back, C., and Unnasch, T. R. (1996). Intraspecific heterogenity of the rDNA internal transcribed spacer in the *Simulium damnosum* (Diptera: Simuliidae) complex. Mol. Biol. Evol. 13: 244-252

Van Baalen, M., and Sabelis, M. W. (1995). The Milker-Killer dilemma in spatially structured predator-prey interactions. Oikos 74:391-400

Vet, L. E. M., and Dicke. M. (1992). Ecology of info chemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37:141-172

Vogler, A. P., and Desalle, R. (1994). Evolution and phylogenetic information content of the *ITS1*- region in the tiger beetle *Cicindela dorsalis.* Mol. Biol. Evol. 11:393-405

Wesson, D. M., McLain, D. K., Oliver. J. H., Piesman, J., and Collins, F. H. (1993). Investigation of the validity of species status of *Ixodes dammini* (Acari: Ixodidae) using rDNA. Proc. Natl. Acad. Sci. U.S.A. 90:10221-10225

Zhang, Z.-Q., and Sanderson. J. P. (1993). Hunger and age effects on searching behavior of three species of predatory mites (Acari: Phytoseiidae). Can. J. Zool. 71:1997-2004

CHAPTER 1

Development of a method for studying genetics of predatory mite *Phytoseiulus persimilis*: Whole genome amplification of Chelex-extracted DNA from a single mite

Abstract

Genetic studies requiring large quantities of high quality DNA are challenging when small arthropods are used because of the limited amount of DNA that can be obtained from an individual organism. We utilized whole genome amplification to amplify DNA from individual predatory mites, *Phytoseiulus persimilis* Athias-Henrot (Acari: Phytoseiidae), for subsequent experiments. Whole genome amplification in combination with chelex extraction of DNA from a single mite provided us with a large quantity of high quality DNA. The DNA from a single mite can be amplified more than ten thousand fold using as little as 0.01 ng of initial quantity. We confirmed the specificity of the amplified DNA by Polymerase Chain Reactions for an ITS nuclear sequence and a 12 S mitochondrial sequence. We found the polymorphisms in the ITS region in different geographical populations like Sicily, California and Netherlands and also potential polymorphisms were associated with 12 S nucleotide sequences in Sicilian populations. We can now use the DNA amplified through this technique for genotyping individual mites to study the genetics of foraging behavior of this predatory mite.

Introduction

Use of DNA sequence information has became a powerful tool in various areas of studies including genetics and evolution. Polymerase chain reaction (PCR) that allows amplification of a specific DNA fragment was a significant addition for the use of DNA sequence in small

organisms where obtaining large quantity of nucleic acid is difficult. To overcome the common problem of limited availability of a quantity of genomic DNA, many researchers have worked to develop methods to amplify the DNA. Such efforts developed a technique generally named as Whole Genome Amplification (Telenius *et al*., 1992; Zhang *et al*., 1992; Cheung and Nelson, 1996). This technique resulted in significant improvements that minimized the constraint of limited genomic DNA. The Whole Genome Amplification (WGA) method, often referred to as multiple displacement amplification (MDA), has been introduced to amplify greater amount of DNA from initial nanogram quantities of template DNA (Dean *et al*., 2002; Lage *et al*., 2002). Amplifying DNA to a sufficiently large quantity for subsequent studies is a potential advantage of WGA. WGA is one of the promising methods of amplifying whole genomes and uses exonuclease- resistant hexamer primers and Φ29 DNA Polymerase (Dean *et al*., 2002). Gorrochotegui and Black (2003) described the optimization and amplification of mosquito DNA using Φ29 Polymerase.

For WGA to be successful, the technique should be simple, reliable and produce high-quality DNA in large quantity. The fidelity and reproducibility of WGA was demonstrated in many clinical specimens like human diseases and for many epidemiological studies (Hughes *et al*., 2004; Luthra and Medeiros., 2004; Leviel *et al*., 2004; Yan *et al*., 2004). Tanabe *et al.* (2003) evaluated the product of WGA by performing PCR and the data showed that the amplified DNA provided sufficient amount of genomic sequence which could be used for genetic analyses as well as for future work. Mai *et al.* (2004) evaluated the WGA for detecting mutations by sequencing hemoglobin genes and found that this technique is reliable in overcoming the limited amount of genomic DNA in clinical applications. Genetic alteration studies using whole genome amplified DNA with comparative genomic hybridization (CGH) showed the accurate copy

number changes and chromosomal abnormalities (Rusakova and Nosek, 2006). Huges *et al.* (2006) used the whole genome amplified DNA for studying chromosomal changes in prostate cancer. Similarly, Hawken *et al.* (2006) conducted the gene marker analysis on both genomic DNA and whole genome amplified DNA from cattle semen samples showed high percent accuracy in examining nearly 10,000 single nucleotide polymorphic markers. All the above studies demonstrate the development and assessment of WGA in terms of the amplification, accuracy and representativeness.

We have been interested in the genetics of foraging behavior in *Phytoseiulus persimilis* Athias Henriot (Acari: Phytoseiidae) a specialist predatory mite on twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). Margolies *et al.* (1997) measured the genetic variation of predators in response to herbivore-induced plant volatiles. This was further confirmed in several behaviors relevant to the foraging of predatory mite by Jia *et al.* (2002). We developed a simple MDA method and tested for a number of molecular markers**.** We used the molecular markers using nuclear ITS and mitochondrial 12S genes in *P. persimilis* (Navajas *et al*., 1998; Kambhampati and Smith., 1995) to validate the method.

Materials and Methods

Mites

Phytoseiulus persimilis used for these experiments were obtained from various geographical origins. Mites from Sicily and Netherlands were obtained either in alcohol or as dried samples and were stored at -20 °C through out the experiments. Mites from the USA were obtained from commercial suppliers, Koppert Biological Systems (Ann Arbor, Michigan) and Rincon-Vitova Insectaries, Inc. USA (Ventura, California) and were maintained on twospotted

spider mites under laboratory conditions of 24-25 °C, 55-60 % RH and 16:8 L: D photoperiod. We used mites from Koppert Biological Systems for optimizing the WGA technique and the rest of the mites from different sources were used for developing molecular markers. Two spotted spider mites, the prey, were maintained on bean leaves under greenhouse conditions. (400 W high-pressure sodium vapor lamps, 16:8 L: D photoperiod and 22-25 °C)

DNA Extractions

Phenol-chloroform extraction of DNA was performed (Sambrook and Russell, 2001) for extracting DNA from groups of mites. Mites from Koppert Biological Systems were used for phenol-chloroform DNA extractions. To obtain high quality genomic DNA, 100 to 120 mites were homogenized in 200 µl of lyses buffer (20 mM TrisHCl pH 8.0, 400 mM NaCl, 5 mM EDTA, 1 % SDS). The homogenate was incubated in 10 µg/ml Proteinase K (Roche) for 30 minutes at 55 °C. One round of standard phenol-chloroform extraction followed by sodium acetate precipitation was performed. Genomic DNA was extracted from single mites by the chelex maceration method (Walsh *et al*., 1991); each individual mite was homogenized separately in a 0.2 ml micro centrifuge tube in 15 µl of 10 % (100 mg /1 ml of water) chelex-100 (Bio-Rad) containing 1µM NaOH. The homogenates were boiled at 94 ºC for 10 minutes followed by 15 min incubation at 75 ºC. The supernatant was used as template DNA to WGA of single mite DNA.

Developing ITS and 12 S molecular markers

Polymerase chain reaction (PCR) comprised of 10 x PCR buffer (Invitrogen), $MgCl_2$ (3.0 mM), each forward and reverse primers (0.2 µM), dNTPs (0.2 mM) and *Taq* Polymerase (0.5 U) in total 20 µl of reaction volume. The thermocycler was set for 94 °C for 5 minutes followed by

36 cycles of 94 °C for 30 seconds, 44 °C for 30 seconds, 72 °C for 1 minute and finally 10 minute elongation at 72 °C. Primers for 12 S (5'TACTATGTTACGACTTATCCTCT3' as forward primer and 5'CCTATTAGTTACATTTAAAATT3' as reverse primer) were selected and modified from previous studies by Kambhampati and Smith (1995).

Primers for ITS1 and ITS2 (5'AGAGGAAGTAAAAGTCGTAACAAG 3' for the 3' end of 18 rDNA and 5'ATATGCTTAAATTCAGGGGG 3' for the 5' end of the 28S) were selected based on the study of Navajas *et al.* (1998). The PCR products were electrophoresed on a 5 % polyacrylamide gel, and stained with 1 % ethidium bromide to visualize the bands. Direct sequencing of PCR products for both directions were made after DNA purification by Zymoclean™ Gel DNA Recovery Kit (Zymo Research). PCR product was cloned into pGEM-T-Easy Vector (Promega) and sequenced. The sequence was analyzed by using software Sequencher (Gen Codes Co.). The sequences for ITS1 and ITS2, and 12 S for *P. persimilis* were compared with previous reports (Jeyaprakash and Hoy, 2002, Navajas *et al*., 1998).

Whole genome amplification/ Multiple displacement amplification

One µl of template DNA (either phenol-chloroform extracted or chelex extracted DNA) was added to sample buffer provided from the manufacturer (Amersham). The sample buffer consisted of 10mM TE pH 8.0 and 100µM exonuclease-resistant thiophosphate modified random hexamers (5'$N_pN_pN_pN_{ps}N_{ps}$N-3'). The reaction mixture consisted of 74mM Tris-HCl (pH 7.5), 100mM KCl, 20mM $MgCl_2$, 10mM $(NH_4)_2SO_4$, 2mMdNTPs, 100µM exonuclease-resistant hexamers and 0.2 units of yeast pyrophosphates and one µl of 8 units Φ29Polymerase (GenomiPhi™ Amplification Kit, Amersham Biosciences) was added to four µl of reaction mixture. Optimal condition for the WGA of single mite was sought by varying the quantity of

template DNA as 1, 0.3, 0.1 and 0.03 ng and also for the polymerase enzyme as 0.3, 0.1 and 0.03 times concentration of the original enzyme provided by the amplification kit. The reactions consisting of template DNA and sample buffer were first denatured by heating to 95 °C for 3 minutes followed by cooling down to 4 °C. The enzyme and reaction buffer were then added to the reaction mixture and incubated at 30 ºC for 15 hours and terminated by heating to 65 °C for 20 min. Following the WGA, amplified DNA was diluted to 100 µl by adding 90 µl of ddH2O to the 10 µl of WGA reaction and one µl of this diluted DNA was used as a template for PCR. Diluted WGA product was stored at -20 °C for subsequent use. WGA product was quantified in Spectrofluorometer using Pico green® dsDNA quantification reagents (Molecular Probe) and the procedure used was in accordance with the manufacturer's protocol. Varying degree of amplification with respect to time of amplification was also measured at 2.5, 5, 10, 15 and 24 hours, respectively. We also checked the specificity of WGA by performing PCR with 12 S and ITS primers. Both genomic DNA and WGA from the same *P. persimilis* individuals were tested for these primer sets. WGA obtained from groups of mites and also from individual mite was made to 100 time serial dilutions and subjected to PCR with the primers mentioned above. Genomic DNA served as the positive control for these experiments. The PCR mixture and the PCR cycling's were same as mentioned above.

Results and Discussion

For population and behavioral genetic studies of any specimen, DNA from individuals is a prerequisite and extraction of high-quality DNA in sufficient quantity is highly challenging in some cases. In the case of the predatory mite, *P. persimilis*, DNA extraction using phenol and chloroform was optimum with at least 100 mites, which was a major constraint in genotyping

individual mites where we have to isolate DNA separately from each individual. Extraction of DNA from a single mite was achieved by macerating individual mites in 10 % Chelex 100. We found that the extraction was more efficient in the presence of NaOH and this may be due to better performance of chelex in alkaline medium. However, in working with individual mite DNA, we found that extraction of DNA was very difficult. The amount of DNA we could extract from chelex maceration was approximately 15 ng /individual. In addition to the above problem we always found that the extracted genomic DNA was subjected to greater amount of degradation for some unknown reason. In combating these problems, we tried to amplify the whole insect genome using Φ29 polymerase enzyme and were successful in standardizing the procedure for amplification of whole mite genome from single individual mite.

The amount of amplification of DNA with respect to their corresponding original template DNA and enzyme concentration were compared (Fig .2). There was significant difference between the initial input DNA's ($f = 7.84$, $df = 4$, $P = <0.0001$) (Fig .2). Amount of amplification with a 0.3 ng template was on par with a 1 ng template, which means the optimal requirement for optimal amplification was 0.3 ng. From the data observed we could tell that the amount of amplification was more dependant on initial input DNA rather than enzyme concentration. However, for optimum amplification a minimum of 0.03 times the diluted concentration of enzyme was essential as we observed no sufficient amplification with any enzyme less than that (Konakandla *et al*., unpublished data). Gorrochotegui and Black (2003) reported the production of large quantities of DNA by using 14 ng of original template mosquito DNA. Dean *et al*. (2001) reported the production of a maximum of 20-30 µg of DNA with 0.03 to 300 ng of initial template DNA. Here we have demonstrated that WGA could be used to achieve unlimited supply of DNA for *P. persimilis* individuals with as little as 0.1 ng of original

template DNA (Fig .2). No amplification was observed if we used the samples without denaturation hence we denatured all the samples which is in accordance with Gorrochotegui and Black (2003) who observed zero amplification without denaturation of template DNA.

When the amplification is subjected to different hours of amplification, more than 50 % amplification was achieved within 5 hours, and in 15 hours the amount of amplification was greater than 95 % (Fig. 3) with less degradation of amplified DNA. If exposed for a longer time, more degradation of the final product was observed; hence exposure of 15 hours of amplification could be the optimal time which was less in our case when compared to Dean *et al.* (2001) and Gorrochotegui and Black (2003) who amplified the DNA for about 18 hours. The WGA amplified DNA of all mites was diluted to 100-fold and stored at -20 °C for future genetic procedures.

For successive utilization, the WGA product should perform similar to that of the unamplified genomic DNA. In testing this with known set of specific primers for 12S mitochondrial genes and ITS ribosomal genes, PCR amplification for both genomic DNA and WGA was found to be similar (Fig. 4 a and Fig. 4 b). However, the amplification of 12 S genes was possible with lower dilutions of WGA compared to ITS genes. One of the possible reasons for such difference might be because of larger copy number of mitochondrial genes. Comparing the sequences of ITS with GenBank sequence a potential polymorphism was observed in the 12 S rRNA sequences among the mites from Sicily (Table 1) while the ITS2 region was highly conserved the ITS1 had potential polymorphism among the individuals from California, Sicily and Netherlands (Fig 1 a).

In conclusion, we have developed a simple method for isolating and amplifying whole gDNA from a single mite by using the combination of chelex and WGA method. The WGA of

single mite was also able to work with the above primers hence we can conclude that amplification of whole mite genome from single mite can be used for further genetic studies. As we were interested in studying the foraging behavior of *P. persimilis*, we now can use the WGA product from each individual mite and can further study the molecular genetics associated with foraging behavior of predatory mite, *P. persimilis.*

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References Cited

Campbell, B. C., Steffen-Campbell, J. D., and Werren, J. H. (1993). Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. Insect Mol. Biol. 2:225-237

Cheung, V. G., and Nelson, S. F. (1996). Whole genome amplification using a degenerate oligonucleotide primer allows hundreds of genotypes to be performed on less than one nanogram of genomic DNA. Proc. Natl. Acad. Sci. U.S.A. 93:14676–14679

Dean, F. B., Nelson, J. R., Gieser, T. L., and Lasken, R. S. (2001). Rapid amplification of plasmid and phage DNA using phi 29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res. 11:1095-1099

Dean, F. B. S., Hosono, L., Fang, X., Wu, A. F., Faruqi, P., Bray-Ward, Z., Sun, Q., Du Zong, Y., Du , J. M., Driscoll, W., Song, S. F., Kingsmore, M., Lasken, E. (2002). Comprehensive human genome amplification using multiple displacement amplification Proc. Natl. Acad. Sci. U.S.A. 99:5261-5266

Gorrochotegui-Escalante, G., and Black, IV. W. C. (2003). Amplifying whole insect genomes with multiple displacement amplification. Insect Mol. Biol. 12:195-200

Hawken, R. J., Cavanagh, J. A. L., Meadows, J. R. S., Khatkar, M. S., Husaini, Y., Zenger, K. R., Clintock, Mc. S., Clintock, Mc. A. E., and Raadsma, H. W. (2006). Technical Note: Whole-Genome Amplification of DNA Extracted from Cattle Semen Samples. J. Dairy. Sci. 89:2217-2221

Huges, S., Yoshimoto, M., Beheshti, B., Houlston, R. S., Squire, J. A., and Eans, A. (2006). The use of whole genome amplification to study chromosomal changes in prostate cancer: insights

into genome-wide signature of preneoplasia associated with cancer progression. BMC Genomics 7:65

Hughes, S., Lim, G., Beheshti, B., Marrano, P., Huang, A., and Squire, J. A. (2004). Use of whole genome amplification and comparative genomic hybridization to detect chromosomal copy number alterations in cell line material and tumor tissue. Cytogenet. Genome. Res. 105:18-24

Jia, F., Margolies, D. C., Boyer, J. C., and Charlton, R. E. (2002). Genetic variation in foraging traits among inbred lines of a predatory mite. Heredity 89:371-379

Kambhampati, S., and Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Mol. Biol. 4:233-236

Lage, J. M., Leamon, J. H., Pejovic, T., Hamann, S., Lacey, M., Dillon, D., Segraves, R., Vossbrinck, B., Gonzalez, A., Pinkel, D. (2003). Whole genome analysis of genetic alterations in small DNA samples using hyperbranched strand displacement amplification and array- CGH. Genome Res. 13**:**294-307

Leviel, K., Olarte, M., and Sullivan, P. F. (2004). Genotyping accuracy for whole genome amplification of DNA from buccal epithelial cells. Twin Res. 7:482-484

Luthra, R., and Medeiros, L. J. (2004). Isothermal multiple displacement amplification: a highly reliable approach for generating unlimited high molecular weight genomic DNA from clinical specimens. J. Mol. Diagn. 6:236-42

Mai, M., Hoyer, J. D., and McClure, R. F. (2004). Use of multiple displacement amplification to amplify genomic DNA before sequencing of the alpha and beta hemoglobin gene. J. Clin. Pathol. 57:637-640

Maeda, T., Takabayashi, J., Yano, S., and Takafuji, A. (1999). Response of the predatory mite,

Amblyseius womersleyi (Acari: phytoseiidae), toward herbivore-induced plant volatiles: variation in response between two local populations. App. Entomol. Zoo. 34:449-454.

Margolies, D. C., Sabelis, M. W., and Boyer, Jr. J. E. (1997). Response of a Phytoseiid Predator to Herbivore Induced Plant Volatiles: Selection on Attraction and Effect on Prey Exploitation. J. insect Behav. 10:695-709

Mayland, H., Margolies, D. C., and Charlton, R. E. (2000). Multiple prey related cues influence residence time of an acarine predator, *Phytoseiulus persimilis,* on prey host plants. Entom. Exp. Apl. 96:245-252

McLain, D. K., Wesson, D. M., Collins, F. H., and Oliver, J. H. JR. (1995). Evolution of the rDNA spacer, *ITS2*, in the ticks *Ixodes scapularis* and *I. pacificus* (Acari: Ixodidae). Heridity 75:303-319

Navajas, M., Lagnel, J., Gutierrez, J., and Boursot, P. (1998). Species- wide homogeneity of nuclear ribosomal *ITS2* sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heridity 80:742-752

Sabelis, M. W. (1981). Biological control of two spotted spider mites using phytoseiid predators. I. Agric. Res. Report. 910 Pudoc, Wageningen, the Netherlands

Sambrook, J., and Russell, D. W. (2001). Molecular Clonong: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory, New York.

Schlotterer, C. M. T., Hauser, A., Von Haeseler., and Tautz, D. (1994). Comparative evolutionary analysis of rDNA ITS regions in *Drosophila.* Mol. Biol. Evol. 11:513-522

Tang, J., Toe, L., Back, C., and Unnasch, T. R. (1996). Intraspecific heterogenity of the rDNA internal transcribed spacer in the *Simulium damnosum* (Diptera: Simuliidae) complex. Mol.

Biol. Evol. 13:244-252

Tanabe, C., Aoyagi, K., Sakiyama, T., Kohno, T., Yanagitani, N., Akimoto, S., Sakamoto, M., Sakamoto, H., Yokota, J., Ohki, M., Terada, M., Yoshida, T., and Sasaki, H. (2003). Genes Chromosomes Cancer 38:168-176

Telenius, H., Carter, N. P., Bebb, C. E., Nordenskjold, M., Ponder, B. A., and Tunnacliffe, A. (1992).Degenerate oligonucleotideprimed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718–725

Rusakova, V., and Nosek, L. (2006). Amplification of genome representative DNA from limited sources with GenomePlex ® WGA technology for use in genetic alterations studies. Nature Methods DOI:10.1038

Vogler, A. P., and Desalle, R. (1994). Evolution and phylogenetic information content of the *ITS1*- region in the tiger beetle *Cicindela dorsalis.* Mol. Biol. Evol. 11:393-405

Walsh, P. S., Metzger, D. A., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material BioTechniques 10:506–513

Wesson, D. M., McLain, D. K., Oliver, J. H., Piesman, J., and Collins, F. H. (1993). Investigation of the validity of species status of *Ixodes dammini* (Acari: Ixodidae) using rDNA. Proc. Natl. Acad. Sci. U.S.A. 90:10221-10225

Yan, J., Feng, J., Hosono, S., and Sommer, S. S. (2004). Assessment of multiple displacement amplification in molecular epidemiology. Biotechniques 37:136-138, 140-143

Zhang, L., Cui, X., Schmitt, K., Hubert, R., Navidi, W., and Arnheim, N. (1992). Whole genome amplification from a single cell: implications for genetic analysis. Proc. Natl. Acad. Sci. U.S.A. 89:5847–5851

Figure 1. PCR product of the region covering ITS1 and ITS2. (a) The samples are from single mites of *P. persimilis* of 3 geographical populations (+ for positive control, C for California, S for Sicily, and N for Netherlands). (b) Structure of ITS1 and ITS2. Small arrows show the locations of the primer

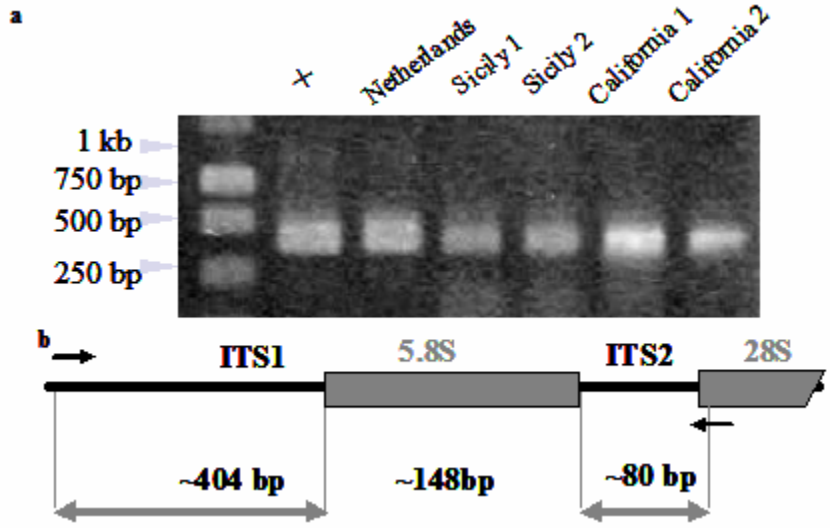

Figure 2. Whole genome amplification with different concentrations of input DNA and enzyme. Each bar represents the amount of DNA amplified from initial template DNA with respective enzyme concentrations (0.3, 0.1 and 0.01x). The data shown are the means of 3 experiments.

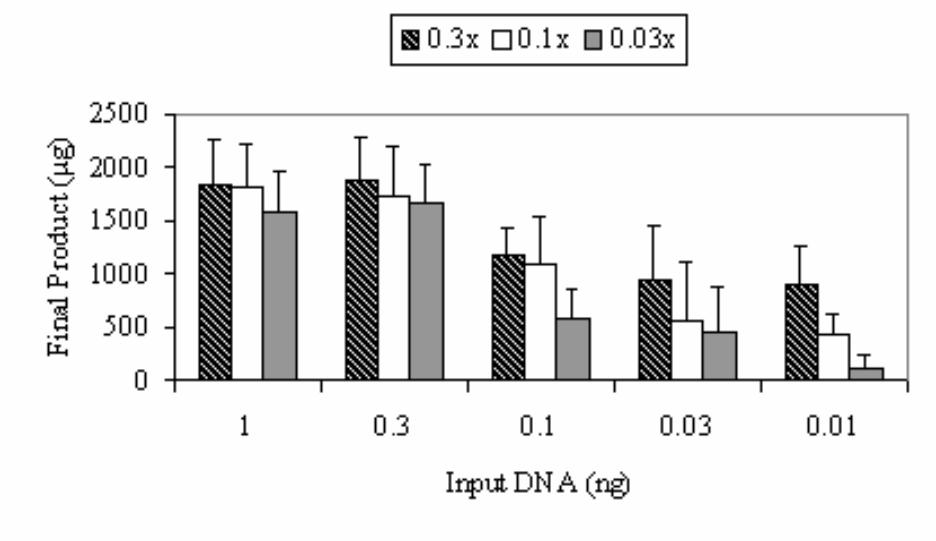

Figure 3. Percent rate of amplification of DNA when subjected to 2.5, 5, 10, 15 and 24 hours of amplification. The data shown are the means of 3 experiments.

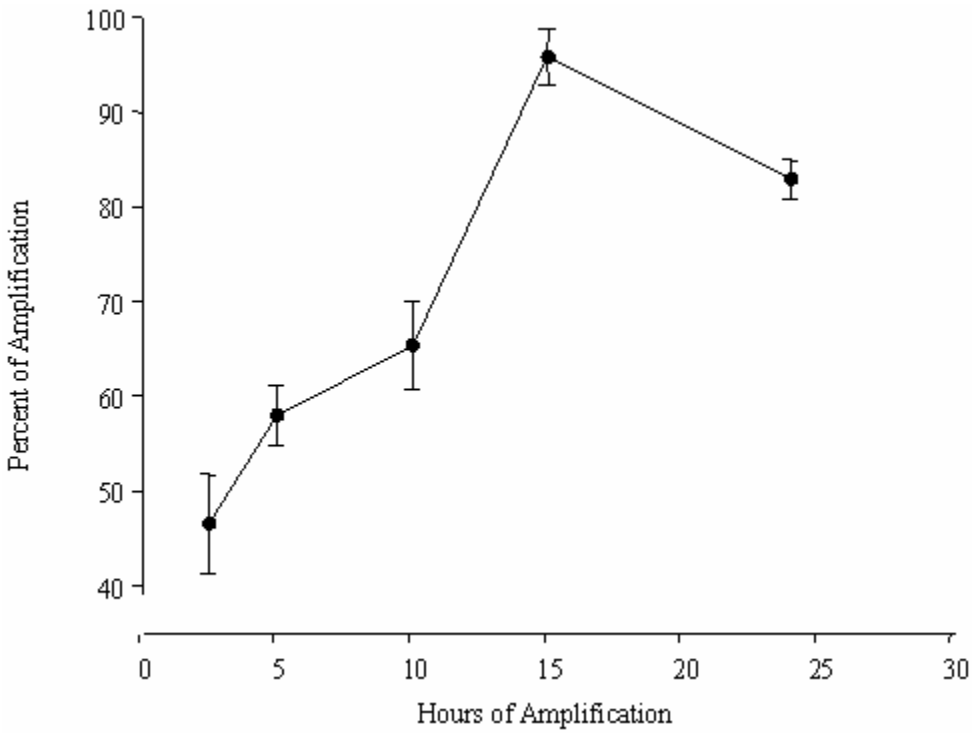

Figure 4. PCR amplification of 12 S by using the template of WGA product from both Phenol-chloroform and Chelex extracted DNA with genomic DNA (+) and negative controls (-) while the bottom gel shows the PCR products with ITS primers comparing WGA from both Phenol-Chloroform and Chelex extracted DNA with Genomic DNA (+) and negative controls. For both sets of primers the WGA's were made into four aliquots (a to d) which differed in concentrations. 'a' is the original WGA diluted 100 times and from this a serial dilution of 100 times was made into b, c and d. Two negative controls are 'a' the negative control using water as template and 'b' the negative control used in the WGA process.

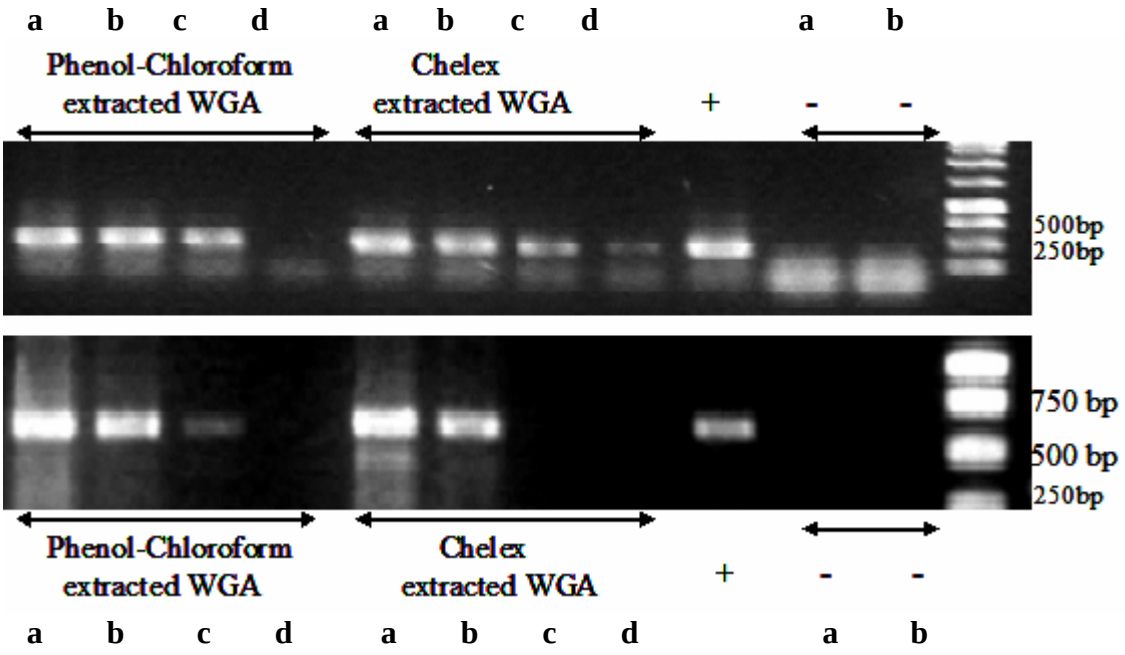

Table 1. Single nucleotide polymorphisms found in 12S sequences of *P. persimilis.* S represents for mites from Sicily.

Individual id	**S2**	**S3**	**S3**	**S4**
***Position**	**16**	**105**	**108**	**270**
Consensus/Polymorphism	**G/A**	**T/G**	**T/C**	**T/C**

CHAPTER 2

The foraging gene *Ppfor* in the predatory mite, *Phytoseiulus persimilis*

Abstract

Phytoseiulus persimilis (Acari: Phytoseiidae) is a specialist predator on tetranychid mites, especially on the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). The foraging environment of the predatory mites consists of prey colonies distributed in patches within and among plants. Previous studies have found that several behaviors related to foraging are governed by genetic factor(s). Therefore, we used molecular techniques to investigate the genes related to variation selected behaviors. We were particularly interested in the resource/prey-dependent dispersal behavior of the predator. The *for* gene is associated with the rover-sitter phenotypes in fruitflies and is known for its role in foraging behaviors in other arthropods like honeybees and ants. We isolated a partial sequence that is presumed to be the orthologue of the foraging (*for*) gene, which we have named *Ppfor* (*Phytoseiulus persimilis* foraging gene). We studied the use of the *Ppfor* gene to develop molecular markers for, and to help understand behavioral phenotypes in different geographical populations of *P. persimilis*.

Introduction

The effect of genes influencing naturally occurring behavioral traits is of great importance for better understanding the behavior and evolution of behavior. Molecular genetics of behavior(s) within and between populations provides the clues about the forces shaping the evolution of behavioral genes. Most complex behaviors are influenced by many genes (polygenic inheritance), but the actual number of genes and the magnitude of their individual effects is

controversial (Falconer, 1981). Complex behaviors in *Drosophila*, the honeybee and mice were influenced by many genes (Singh and Pandey, 1993; Lapidge *et al*., 2002; Hofstetter *et al*., 1995) However, many behavioral changes in humans, mice and *Drosophila* have been identified as effects of single genes or loci (Hall, 1994; McKlusick, 1994; Takahashi *et al.,* 1994; Takahashi *et al.,* 2000).

Sokolowski (1980) described Rover-Sitter foraging strategies in natural populations of *Drosophila melanogaster* which differ in the length of distance traveled in search of food. Rovers travel long distance in search of food and leave the food source quickly to find others, whereas sitters travel short distances and do not leave the food source unless it is depleted. These phenotypic differences were attributed to a single major gene called *foraging (for)* (de Belle *et al.*, 1989). The rover allele (for^R) is dominant to the sitter allele (for^s) (de Belle and Sokolowski, 1987). This *for* gene was found to be the gene previously described as *dg2* and encodes for one of two PKG genes in *D. melanogaster* (Osborne *et al*., 1997; Kalderen and Rubin, 1989).

Ben-Shahar *et al.* (2002) reported an orthologue of *for* (Orthologs are homologs which are produced by speciation which look structurally similar but may or may not display similar activity) in the honey bee, *Apis mellifera,* which he called *Amfor.* This gene was demonstrated to play a role for PKG in the behavioral transition from nurse to forager. Nurse bees are younger bees, which distribute food within the hive, have lower PKG activity levels and lower abundance of *Amfor* RNA compared to the older forager bees. The differences in the levels of *Amfor* RNA and PKG levels lead to the behavioral transition irrespective to the age of bees. Ingram *et al.* (2005) found that the expression of *for* (again *for* orthologue) in red harvester ants, *Pogonomyrmex barbatus,* was associated with foraging behavior of these ants; callow

workers (young adults) brains had significantly higher levels of *for* mRNA than foragers (older adults) and higher PKG activity was observed in callows than foragers. In the nematode worm, *Caenorhabditis elegans, elg-4,* which encodes for PKG, has been shown to influence food-related behaviors (roaming and dwelling). These behaviors again differ in their distance traveled while feeding. Roaming is defined by long distances of uninterrupted locomotion and dwelling involves short distances and frequent stops. Knock out experiments of *egl-4* showed that decreasing PKG causes an increase in roaming behavior on food (Fujiwara *et al.,* 2002). Though there are differences in function, the foraging behavior in all these organisms revolve around the activities of PKG.

Margolies *et al.* (1997) documented intra-population genetic variation in several traits related to the foraging efficiency of the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). These included predator response to spider mite-induced volatiles and other foraging traits in the tritrophic interaction. Jia *et al.* (2002) confirmed the above findings through quantitative genetic studies. *P. persimilis* differ in foraging efficiency in terms of response to plant volatiles and dispersal. The foraging strategies observed in mites were similar to the behavioral phenotypes of *Drosophila* as discussed above. We report here the isolation of a foraging gene in *P. persimilis* similar to the foraging gene in other arthropods. We also show the existence of polymorphism associated with this gene.

Materials and Methods

Bean plants (*Phaseolus lunatus* L, cv. 'Sieva') served as host plants for spider mites in all experiments. Beans were grown in the greenhouse under 400 W high-pressure sodium vapor lamps on a 16:8 L: D photoperiod and 22-25 °C. The population of *Tetranychus urticae* that we

used as prey were reared on greenhouse-grown bean plants in a rearing room under the same light and temperature conditions. Predatory mites (*Phytoseiulus persimilis*) for this experiment were originally obtained from commercial suppliers, Koppert Biological System U.S.A (Ann Arbor, Michigan) and Rincon-Vitova Insectaries, Inc. U.S.A (Ventura, California). The population was maintained on spider mite-infested bean leaves at 24-25 °C, 55-60 % RH and a 16:8 L: D photoperiod. We established base populations from these colonies. Other geographical populations of predators were obtained from Netherlands and Sicily. Mites obtained from the U.S.A. are hereafter designated as the 'Michigan population' and the 'California population'.

To design degenerate primers for the PCR amplification of the genes homologous to the *Drosophila for* gene (dg2-T1), we fetched PKG genes in different organisms like nematodes, insects, and mammals. The amino acid sequences were aligned using Vector NTI software in order to design degenerate primers. The regions containing low degeneracy amino acids and the highly conserved sequences were selected for designing primers. Genomic DNA of predatory mites was extracted from individual mites by performing chelex extraction (Walsh *et al*., 1991) where each mite is placed in a 0.2 ml micro centrifuge tube and 5 µM chelex 100 (Bio-rad) and 5 µl of 1 µM NaOH and homogenized with pipette tips. The homogenate was subjected to 95 °C for 15 minutes followed by 60 °C for 10 minutes. Separately, high-quality genomic DNA was isolated from a pooled 100-200 mites. The group of mites was homogenized in lysis buffer (20 mM TrisHCl pH 8.0, 400 mM NaCl, 5 mM EDTA, 1% SDS) and 10 µg/ml Proteinase K (Roche) was added to the homogenate. The mixture was then incubated for 30 minutes at 55 °C. One round of phenol-chloroform extraction was followed by precipitation of DNA with 0.3 M sodium acetate and absolute ethyl alcohol. The pellet was washed with 70 % ethanol and suspended in 20 µl of water. Polymerase Chain Reaction (PCR) were carried in 20 µl reaction mixture

containing 10 x PCR buffer (Invitrogen), $MgCl_2$ (3 mM), each forward and reverse primers (0.2 µM), dNTPs (0.2 mM) and *Taq* Polymerase (0.5 U). The PCR cycling 94 °C for 5 minutes followed by 36 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1 minute and finally 10 minute elongation at 72 °C. All reactions were visualized on 1 % agarose gels stained with ethidium bromide.

For further extension of the *for* gene in *P. persimilis* in both the 5' and 3' directions, we performed vectorrette PCR as explained in Wang *et al.* (2006). Once a fragment of the *for* gene in *P. persimilis* was obtained, the nucleotide sequences were aligned using the Clustal W 1.5 program (Thompson *et al*., 1994) and the alignment was manually corrected and the phylogenetic analysis was performed using PAUP 4b2 (Swofford, 1998). We aligned and compared the sequence of mite *for* gene with *Drosophila*, the honeybee, the nematode *C.elegans*, the silkworm, mice and humans and the phylogenetic tree was constructed.

From the amplified *for* gene sequence specific primers (forward: 5'GGCGTGGGCGGCTTCGGCAG3**';** reverse: 3'CTTCGCGAAGAAGCTGACGT 5') were designed to search for polymorphism in predatory mites, both among populations and among individuals. PCR products were cloned onto vector pGEM-T (Promega) vector and sequenced and studied for polymorphism using Sequencher (Gen Codes Co.) software. We also did a genomic DNA southern blotting to check the copy number of the *for* gene in *P. persimilis.* DNA was digested with *EcoR*I, and the resulting fragments were separated on 0.8 % agarose gel. After transfer to nylon membranes, blots were hybridized with a [^{32}P]dCTP-labeled probe. The probe was a 446 bp containing *for* gene of *P. persimilis*.

As the *for* gene has a predominant PKG domain, we were interested in investigating the role of PKG in particular to the foraging behaviors of *P. persimilis,* for which we injected

predatory mites with 8-Br-cGMP and 8-Br-cAMP. Water and non-injected mites served as controls. Predatory mites were injected with about 10 nL of the 10µM 8-Br-cGMP, 10µM 8-Br-cAMP, respectively, by using micropipettes. All predators used for experiments were gravid females that were left unfed for a period of 2 hours before the injections. This minimized the variation in hunger level and age. The needles were pulled with a model P-87 Flaming/ Brown micropipette puller using borosilicate glass with O. D: 1.0 mm and I. D: 0.50 mm. After the needles had been loaded with the treatments, the contents were injected mostly into the abdomen of the adult predators by using PMI-200 Pressure Microinjector (Dagan).

After the injections, the mites were found to be in trauma for ~ 2-3 hrs after which most recovered. After recovery, we tested for consumption efficiency, oviposition and dispersal behavior. We tested the consumption rate and oviposition rate of injected predatory mites by performing an assay according to Jia *et al.* (2003). A bean leaf disk consisting of eggs of *T. urticae* was placed in a vial and then the injected predator was released into the vials. Each predator was provided with 40 *T. urticae* eggs and the vials were placed at controlled environmental conditions (24-25 °C, 55-60 % RH and 16:8 L: D photoperiod) and left for 24 hours. Consumption was tested based on the number of remaining prey eggs. At the same time the number of eggs deposited by each predator was recorded. To test the dispersal behavior of predatory mites, we followed the bioassay developed by Maeda and Takabayashi (2001). The experimental set up consisted of a square of *T. urticae*-infested leaf connected to a bean leaf disk via a paraffin bridge. The whole set up was placed on moist cotton in a plastic Petri dish. The Petri dishes were then placed in a wind tunnel in a climate-controlled room (25 ± 2 °C, 50 - 70 % RH). The leaf square was placed upwind of the leaf disc at 45° angle. Prior to the day of the test, we introduced 5 adult females of *T. urticae* on an uninfested disk and allowed them to oviposit

for 24 hours. The next day we gently removed the adults with a brush. The prey- infested squares were used for trapping the dispersing predators. The injected predators were placed on the leaf disks and counted for the dispersal rate every 15 minutes for 2 hours.

Results and Discussion

Based on the highly conserved amino acid sequences of PKG protein from *Drosophila,* honeybee, mosquito, silkworm, nematode, mice and human using degenerate primers 5'CARCARCARCAYATHATG 3' and 5' CCRCARAANGTCCANGTYTT 3', about 446 bp fragment of *for* gene was amplified in *Phytoseiulus persimilis* which was named as *Ppfor* (*Phytoseilus persimilis for*). By adopting vectorrette PCR using the internal and external specific primers we could extend about 282 bp of *Ppfor* at the 5' end and altogether the amplified *Ppfor* was approximately 728 bp. Fig 2.1(a) shows the amplified region of *Ppfor* with respect to the *dg2* of *Drosophila (*Fig .5(b)). Alignment of the deduced amino acid sequence of *Ppfor* with those of *Drosophila dg2*, honeybee *Amfor* and other organisms reveals an overall organization of protein kinase domain. The deduced amino acid sequence shares more than 80 % overall identities with *Drosophila dg2* and honeybee *Amfor,* respectively. *Ppfor* sequence was highly conserved with other members of *for* gene in diverse organisms in the multiple sequence alignment (Fig .6). Phylogenetic analysis performed on the software PAUP* 4b for generating distance tree suggests that the *Ppfor* is orthologous to the *Drosophila for* (dg2-T1) (Fig .7).

The specific primers routinely amplified 675bp fragment of *Ppfor* from individuals (n = 52). In analyzing the clones of the *Ppfor* we found a high degree of polymorphism in the region of *Ppfor* between individuals and among populations (Table .2). Altogether we sequenced 154 clones, both from individuals and two different populations. Within the fragment of *Ppfor* we

found 13 different alleles in the California population, 10 different alleles in the Michigan population, and 4 different alleles in various individuals belonging to Sicily, California and New Zealand. These polymorphic sequences can be used as genetic markers in *P. persimilis*. Copy number of *Ppfor* was verified by Southern blot analysis on *EcoR*I-digested genomic DNA with a fragment of *Ppfor* probe. The presence of a single band was detected and was about 4kb suggesting that *Ppfor* in *P. persimilis* is a single copy gene (Fig .7).

The amplified fragment of *Ppfor* was the protein kinase domain. The sequence analysis of *Ppfor* demonstrated that it is likely an orthologous gene to *dg2*, which encodes a *Drosophila* cGMP dependant protein kinase (PKG) (Osborne *et al*., 1997). Subtle differences in PKG are sufficient to produce significant differences in larval foraging behavior (Sokolowski, 1998). The roles of PKG in cell signaling are not well understood (Wang and Robinson, 1997). One way by which PKG activities were increased in the cell is by activating guanyl cyclase via nitric oxide, thus increasing the intracellular level of cGMP. Even though PKG is not involved in all intracellular cGMP signals, it is thought to be a major effector of cGMP.

Injection experiments using 8-Br-cGMP and 8-Br-cAMP showed no significant difference in consumption and oviposition ($f = 0.44$, $df = 3$, $P = 0.72$; $f = 0.73$, $df = 3$, $P = 0.54$) but the 8-Br-cGMP-treated mites were significantly different from 8-Br-cAMP, water and non-injected mites ($f = 7.43$, $df = 3$, $P = 0.0045$; Fig .8). The 8-Br-cGMP-treated mites dispersed quickly in comparison with other treatments, which suggests that PKG has some role in dispersal behavior of predatory mites. PKG function may be important for feedback control of foraging. Feeding is influenced by the fullness of the gut which sends the signals to the brain. PKG may act to increase neural excitation, play a role in long-term potentiation (Zhuo *et al*., 1994) or it may be involved in output of behavioral response.

Identification of the *Ppfor* gene and documentation of polymorphic patterns in DNA sequence provides a critical step towards a comprehensive functional characterization of this gene in future studies. Such studies should include determining if differences in *Ppfor* expression are associated with changes in foraging behavior. Thus, a better understanding of foraging behavioral polymorphism in relation to genetic variation in the presumed foraging gene in *P. persimilis* is needed. Further more association of PKG activity or *Ppfor* expression levels with the foraging behavior can be examined to understand the molecular basis of the behavior.

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References Cited

Ben-Shahar, Y., Robichon, A., Sokolowski, M .B., and Robinson, G. E. (2002). Influence of gene action across different time scales on behavior. Science 296:741-744

Ben-Shahar, Y., Leung, H. T., Pak, W. L., Sokolowski, M. B., and Robinson, G. E. (2003). cGMP-dependant changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. J. Exp. Biol. 206:2507-2515

Bhat, G., Mahesh, V.B., Aguan, K., and Brann, D.W. (1996). Histochemical localization of nitric oxide neurons in the hypothalamus: Association with gonadotropin-releasing hormone (GnRH) neurons and co-localization with N-methyl-D-aspartate (NMDA) receptors. Neuroendocrinology 64:93–102

Coyne, J. A. (1992). Genetics and Speciation. Nature 355:511-515

Day, D. E., Rhinehart, E. K and Bartness, T. J. (2005). Role of NPY and its receptor subtypes in foraging, food hoarding and food intake by Siberian hamsters. Am. J.Physiol. Regul. Integr. Comp. Physiol. 289:29-36

de Belle, J. S., Hilliker, A. J., and Sokolowski, M. B. (1989). Genetic localization of foraging (*for*): A major gene for larval behavior in *Drosophila melanogaster***.**Genetics 123:157-163

de Bono, M., Tobin, D. M., Davis, M. W., Avery, L., and Bargmann, C. I. (2002). Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. Nature 419:899-903

Fujiwara, M., Sengupta, P., and McIntire, S. L. (2002). Regulation of body size and behavioral state of *C.elegans* by sensory perception and the EGL-4-cGMP-dependant protein kinase. Neuron 36:1091-1102

Falconer, D. S. (1981). Introduction to Quantitative Genetics Ed.2. Longman. Newyork

Gudermann, T., Schoneberg, T., and Schitz, G. (1997). Functional and structural complexity of signal transduction via G- protein coupled receptors. Annu. Rev. Neurosci. 20:399-427

Hall, J. C. (1994). The mating of a fly. Science 264:1702-1714

Hofstetter, J. R., Mayeda, A. R., Possidente, B., and Nurnberger, J. I. Jr. (1995). Quantitative trait loci (QTL) for circadian rhythms of locomotor activity in mice. Behav. Genet. 25:545-56

Ingram, K. K., Oefner, P. and Gordon, M. D. (2005). Task- specific expression of the foraging gene in harvester ants. Molecular Ecology 14:813-818

Jia, F., Margolies. D. C., Boyer, J. C., and Charlton, R. E. (2002). Genetic variation in foraging traits among inbred lines of a predatory mite. Heredity 89:371-379

Kalderon, D., and Rubin, G. M. (1989). cGMP-dependant protein kinase genes in*Drosophila*. J. Biol. Chem. 264:10734-10748

Kalra, S.P. (1997). Appetite and body weight regulation: is it all in the brain? Neuron 19: 227–230

Kuo-Dong Yih, Hsu- ChaoTein and Chneg-Juei Tang. (2001). Role of hypothalamic neuropeptide Y (NPY) in the change of feeding behavior induced by repeated treatment of amphetamine. Life Sciences 70:243-251

Lapidge, K. L., Oldroyd, B. P., and Spivak, M. (2002). Seven suggestive quantitative trait loci influence hygienic behavior of honey bees. Naturwissenschaften 89:565–568

Lewis, W. J., Vet, L. E. M., Temlinson, J.H., van Lenteren, J.C., and Papaj, D.R. (1990). Variations in parasitoid foraging behavior: Essential element of a sound biological control theory. Environ. Entomol. 19:1183-1193

Maeda, T and Takabayashi, J. (2001). Patch-Leaving Decision of the Predatory Mite

Amblyseius womersleyi (Acari: Phytoseiidae) Based on Multiple signals from Both Inside and Outside a prey patch. J. Insect. Behav. 14:829-839

Margolies, D. C., Sabelis, M. W., and Boyer, Jr. J. E. (1997). Response of a Phytoseiid Predator to Herbivore Induced Plant Volatiles: Selection on Attraction and Effect on Prey Exploitation. J. Insect. Behav. 10:695-709

Mayland, H., Margolies, D.C., and Charlton, R.E. (2000). Multiple prey related cues influence residence time of an acarine predator, *Phytoseiulus persimilis,* on prey host plants. Entom. Exp. Apl. 96:245-252

McKlusick, V. A. (1994) *Mendelian Inhedance in Man: A Catalog of Human Genes and Genetic Disorders.* Johns Hopkins University Press, Baltimore

Osborne, K, A., Robichon, A., Burgess, E., Butland, S., Shaw, R. A., Coutland, A., Pereria, H. S., Greenspan, R. J., and Sokolowski, M. B, (1997). Natural behavioral polymorphism due to cGMP-dependant protein kinase of *Drosophila.* Science 277: 834-836

Singh, B. N and Pandey, M. (1993). Evidence for additive polygenic control of pupation height in *Drosophila ananassae*. Heriditas 119:111-116

Sisodia, S and Singh, B. N. (2005). Behaviour genetics of *Drosophila*: Non-sexual behaviour. J. Genet. 84:195-216

Sokolowski, M. B., (1980). Foraging strategies of *Drosophila melanogaster*: A chromosomal analysis. Behav. Gen. 10:291-302

Sokolowski, M. B., Pereira, H. S., and Hughes, K. (1997). Evolution of foraging behavior in *Drosophila* by density- dependent selection. Proc. Natl. Sci. U.S.A. 94:7373-7377

Sokolowski, M. B. (1998). Genes for Normal Behavioral Variation: Recent Clues from Flies and Worms. Neuron 21:463-466

Sokolowski, M. B. (2003). NPY and the Regualtion of Behavioral Development. Neuron 39:6-8

Strader, C. D., Fong, T. M., Total, M. R, Underwood, D and Dixon, R. A. (1994). Structure and function of G-protein coupled receptors. Annu. Rev. Biochem. 63: 01-132

Swofford, D. L. (1998). PAUP*. Phylogenetic analysis using parsimony (* and other methods). Ver 4b. Sinauer Associates, Sunderland, MA

Takahashi, J. S., Pinto, L. H., and Vitaterna, M. H. (1994). Forward and reverse genetic approaches to behavior in the mouse. Science 264:1724-1732

Takahashi, A., Tsaur S-C., Coyne, J.A., and Wu C-I. (2001). The nucleotide changes governing cuticular hydrocarbon variation and their evolution in *Drosophila*. Proc. Nat. Acad. Sci. U.S.A. 98:3920–3925

Van Baalen, M., and Sabelis, M. W. (1995). The Milker-Killer dilemma in spatially structured predator-prey interactions. Oikos 74:391-400

Walsh, P. S., Metzger, D. A., and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material BioTechniques 10:506–513

Wang, X., and Robinson, P.J. (1997). Cyclic GMP-dependent protein kinase and cellular signaling in the nervous system. J. Neurochem. 68:443–456

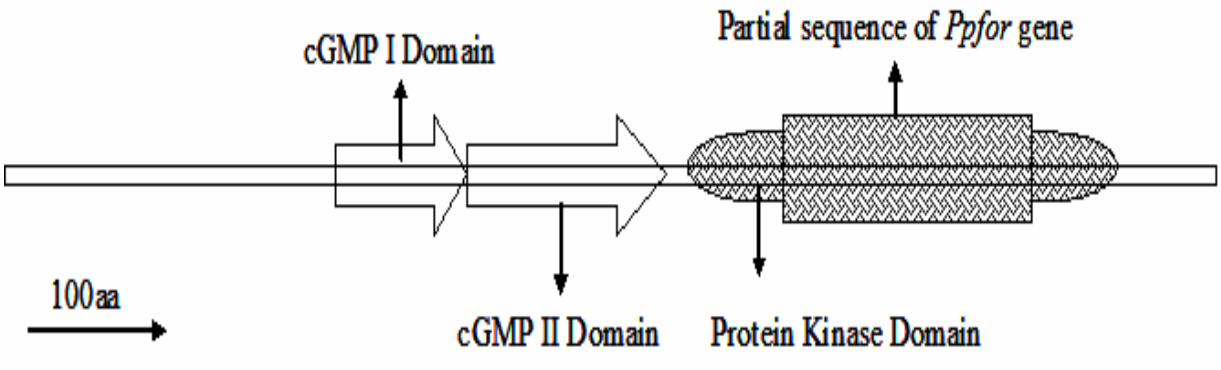
Wang, J., Miller, A. T and Park, Y. (2006). Technical note on Development of multiple dominant markers by using Vectorette PCR-based non-radioactive transposable element display. Molecular Ecology Notes. In Press

Wu, Q., Wen, T., Lee, G., Park, J. H., Cai, H. N and Shen, P. (2003). Developmental control of foraging and social behavior by the. *Drosophila* neuropeptide Y-like system Neuron 39:147-161

Zhuo, M., Ylinghe, H., Schultz, C., Kandel, E. R., and Hawkins, R. D. (1994). Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature 368:635-639

Figure 5. (a) Structural representation of *Drosophila for* gene (934 aa) showing different functional domains. The filled box within the protein kinase domain represents the part of *Ppfor* gene amplified in predatory mite. (b) Nucleotide Sequence of *Ppfor* with two 126bp & 112 bp introns. The sequence also shows the forward and reverse primers used to study the polymorphism. Note that the length of *Ppfor* amplified is also shown.

(a)



(b)

```
          (Forward primer)
        G  V  G  G  F  G  R                                  (126 bp Intron)
  1     GGCGTGGGCGGCTTCGGCAGGgtgagtagcctttaggtttatctcggtggaactccgcgtttcgctcttcattctgttgcggtgtgcttccctcgagcgc

                                                          V  E  L  V  Q  P  V  N  D  P  S  R  S  F  A  L  K
101     ctgcgccacgacccgatgtcattgaactcacgcgaaatttccgccgctcagGTTGAGCTCGTCCAGCCCGTCAACGATCCGAGCCGATCGTTCGCTCTCAA

          V  M  K  K  A  Q  I  V  E  T  R  Q  Q  Q  H  I  M  S  E  K  Q  I  L  V  E  T  N  C  D  F  V  I  K
202     AGTCATGAAGAAAGCGCAGATTGTGGAAACTCGACAGCAGCAGCACATCATGTCCGAAAAGCAGATTCTCGTCGAGACGAACTGTGATTTCGTTATCAAGC

         L  Y  K  T  F  K  D  T  K  Y  L  Y  M  L  L  E  A  C  L  G  G  E  L  W  T  I  L  R  D  R  G  N  F  D
303     TGTACAAAACGTTCAAAGACACGAAGTATCTCTACATGCTTCTCGAAGCCTGCCTCGGAGGAGAACTCTGGACGATCCTGAGGGACCGCGGGAATTTCGAC

         D  S  T  T  R  F  Y  T  A  C  V  L  E  A  F  D  Y  L  H  S  R  N  I  I  Y  R  D  L  K  P  E  N  M
404     GACTCCACAACGCGCTTCTACACAGCTTGTGTTCTGGAGGCGTTCGATTATCTGCATTCTCGCAATATCATTTACCGGGATTTGAAGCCTGAAAACATGCT

         L  L  D  Q  R  G  Y  I  K                                  (112 bp Intron)
505     CCTCGACCAACGCGGCTACATCAAGgtgagcagaatcgaaacgtcccctcgctacggtccggtaggcatccgaagctttctcggtgaatcattccactgcc

                                                    L  V  D  F  G  F  A  K  K  L  T  S
606     gtcgccctgagtgagttcctcgtttatttttccctagCTCGTCGATTTCGGCTTCGCGAAGAAGCTGACGTCCG
                                                    (Reverse primer)
```

Figure 6. *Ppfor* alignment with *for* gene of different organisms including other insects and vertebrates

Figure 7. Phylogenetic tree showing the evolutionary relationship of *for* gene in different organisms. Note that the *Ppfor* is placed in the same clade with *Drosophila for* gene. Numbers indicates percent values in 1000 bootstrapping in distance tree constructed in PAUP4b2

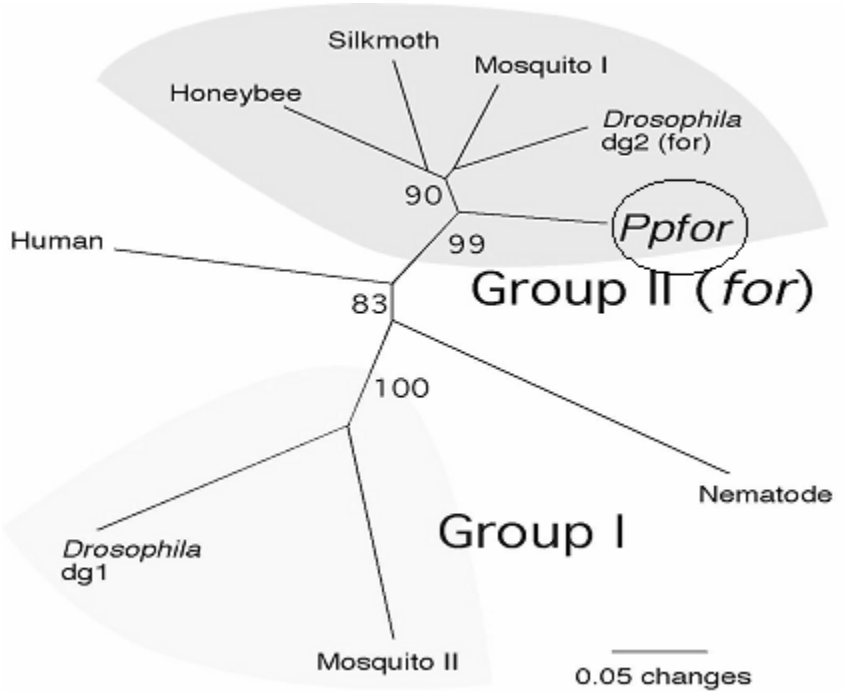


Figure 8. Gel picture showing the polymorphism among different individuals of *P. persimilis.* K21 to K27 are the individual identities of mites belonging to the Michigan population

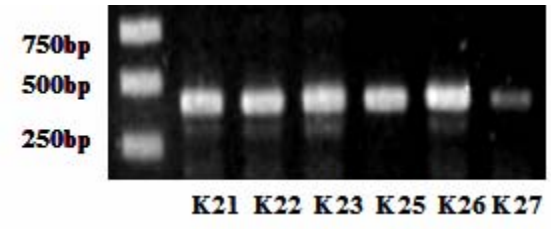


Figure 9. Southern blot showing the *Ppfor* at ~ 4kb

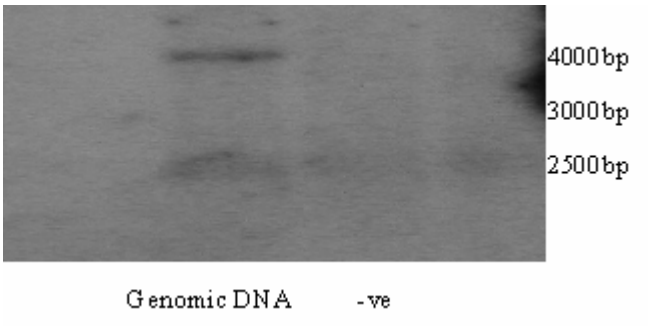


Figure 10. Dispersal of predatory mites under different treatments. The data shown is mean of 4 experiments and n=5 for each experiment

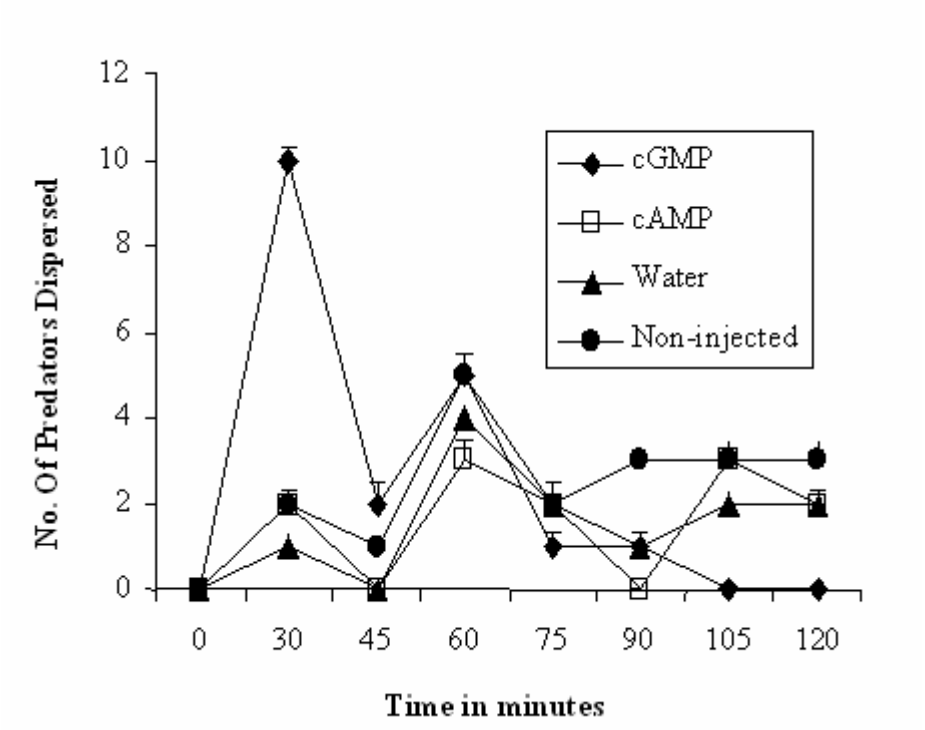


Figure 11.Consumption and oviposition of predatory mites under different treatments (n = 10 for each treatment)

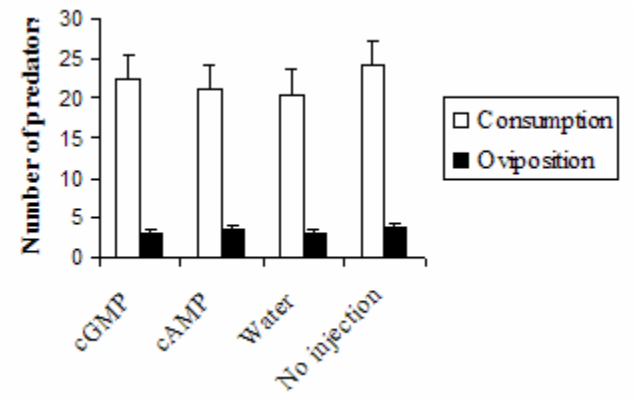


Table 2. *Polymorphism* in the fragment of *for* gene (after forward primer to 360bp) within different populations like California, Detroit and polymorphism between individuals of different populations. *Numbers for nucleotide position are counted immediately after the forward primer. G11 (Kop) is the consensus, A01, A02 are Sicily individuals, A04 is New Zealand individual and A05 is California individual

		Nucleotide posiiton*																									
		13	45	51	63	64	70	113	136	165	180	218	221	224	227	230	270	280	288	292	331	335	341	355	356	359	360
	A08-064	T	C	A	T	G	C	C	A	C	A	G	G	G	C	C	C	A	A	C	C	C	A	C	G	C	C
California population	A01-031																T										
	A02-016																T										
	A03-031																T										
	A04-032			G					G	T							T										
	A05-047																T										
	A06-048	C															T										
	A07-063										G						T										
	A09-079											A		A	T		T	G									
	A10-080	C															T										
	A12-096														T	T	T										
	A13-097												A			A	T										
	B01-013																T										
Michigan population	G05-035																T										
	G06-036																T										
	G07-051	C															T										
	G08	C															T		G								
	G09	C															T										
	G10	C															T										
	G11																T										
	G11-083							G									T										
	G12-084	C															T										
	G13																T										
	F12-086																T						G				
Individuals	A01		A																						C	T	T
	A02																				T	A		A			
	A04				C	A	A										T			T							
	A05																T										

Table 3. *Polymorphism* in the fragment of *for* gene (from 360 bp to reverse primer) within different populations like California, Detroit and polymorphism between individuals of different populations. *Numbers for nucleotide position are counted immediately after the forward primer. G11 (Kop) is the consensus, A01, A02 are Sicily individuals, A04 is New Zealand individual and A05 is California individual

		Nucleotide posiiton*																							
		364	386	392	407	442	453	455	496	499	500	502	536	539	542	580	585	598	599	600	603	604	606	607	610	642
	A08-064	G	C	A	C	T	C	T	G	G	C	A	C	C	T	A	C	G	A	G	C	C	C	G	T	A
California population	A01-031																									
	A02-016																									
	A03-031																									
	A04-032																									
	A05-047			T	T	A	G	G																		
	A06-048															C										
	A07-063															C										
	A09-079		T																							G
	A10-080															C										G
	A12-096																									G
	A13-097																									
	B01-013																									G
Michigan population	G05-035																								C	
	G06-036																					T				
	G07-051																									
	G08																									
	G09																									
	G10								C	A	A	G	T	A	G											
	G11																									
	G11-083																	T	T	C	T	T				
	G12-084																T									
	G13																									
	F12-086																					T	T	C		
Individuals	A01	A																								
	A02																									
	A04																									
	A05																									

SUMMARY AND CONCLUSIONS

Foraging for food is a crucial behavior for animals. Like all other behaviors it requires the interaction of many components. Nonetheless, it turns out that in some animals, at least foraging behavior can be altered by a single gene. Many ecologists argue that foraging attributes are targets of natural selection and must therefore be inherited (Pulliam, 1981). Although foraging behavior has been studied in detail by behavioral ecologists, little is known about the heritable basis of this trait and under what conditions individuals differ in behaviors that contribute to fitness.

Natural population of any organism maintains large amount of genetic variation and this variation thus leads to evolution (Sisodia and Singh, 2005). There exists genetic variation in the case of foraging behavior(s) of the predatory mite, *P. persimilis,* as influenced by interactions with *T. urticae* and plants. Knowledge of the behavior induced in *P. persimilis* by plant-producing chemicals has been applicable for pest management. The information linking *P. persimilis*, search behavior, foraging efficiency and *P. persimilis - T. urticae* population dynamics will allow us to best utilize *P. persimilis* for control of *T. urticae.* Thus, understanding the genetics of the foraging behavior has implications for better utilization of *P. persimilis* as a biological control agent. Using molecular technology to study arthropod genetic diversity and how it relates to behavior is important as no molecular approach has been undertaken until our work with *P. persimilis*. However, DNA is the first limiting factor in performing genetic studies of these predators because of the size of the mite and problems with isolation of intact DNA without degradation. To combat this problem we employed Whole genome amplification technique and were successful in acquiring unlimited supply of DNA from a little input template.

The template we used was much lower than what Dean *et al.* (2002) and Gorrochotegui and Black (2003) used for their studies with human and mosquito samples, respectively. Despite this, we were able to amplify huge amounts of final product. The entire amplified DNA was stored at -20° C and could be used for any genetic studies in future (Dean *et al.*, 2002).

We were also successful in cloning the presumed orthologue of the *for* gene in *P. persimilis* which we named *Ppfor*. By using a robust PCR, we developed a number of potential molecular markers within various populations from Michigan, California, Sicily and New Zealand. The development of polymorphic markers provides a valuable tool for studying mite ecology and genetics, such as identification of strain-specific markers for tracking allele frequency changes and quantitative trait loci (QTL) analysis for biologically important or evolutionarily interesting traits.

The polymorphic markers that we developed for *Ppfor* supports that the mites differ in their genetic makeup for the foraging gene, *Ppfor*. To ensure that genetic variation results in behavioral differences in foraging/dispersion further research needs to be done via expression analysis of *Ppfor* in selected individuals for various foraging traits. Also to identify the individual genes involved in the behavioral response, cDNA micro arrays could be used to identify the candidate genes and then could assess the predatory lines in these genes for behavioral confirmation. Thus the contribution of gene(s) for the complex polygenic behavior of foraging / dispersion might be determined.

Subtle changes in the behavior of natural enemies can lead to significant differences in population dynamics, both in theory and in reality (Hassell & May, 1985; Pels *et al*., 2002; Murdoch *et al*., 1996). The knowledge of the behavioral basis of population ecology helps us to

predict the responses of predator-prey systems to novel conditions or to deliberate manipulations. Thus, documentation of genetic variation in foraging behavior is also important for selection and application of natural enemies in biological control. To summarize, this research is expected to: 1) help understand the link of variation of foraging behavior in predatory mites to gene(s) involved in elicitation of that behavior, 2) better understand the role of dispersal and response of predator to herbivore-induced volatiles in *P. persimilis* predator-prey dynamics, and 3) assess the possibility of genetic improvement of *P. persimilis* as a biological control agent.

References cited

Dean F. B. S., Hosono, L., Fang, X., Wu, A. F., Faruqi, P., Bray-Ward, Z., Sun, Q., Du Zong, Y., Du, J. M., Driscoll, W., Song, S. F., Kingsmore, M., Egholm, Lasken. (2002). Comprehensive human genome amplification using multiple displacement amplification. Proc. Natl. Acad. Sci. U.S.A. 99:5261-5266

Gorrochotegui-Escalante, G and Black IV, W. C. (2003).Amplifying whole insect genomes with multiple displacement amplification. Insec. Mol. Biol. 12:195-200

Hassell, M. P., and May, R. M. (1985). From individual behaviour to population dynamics. In: Behavioural ecology (Sibly RM, Smith RH, eds). Oxford: Blackwell:3-32

Pulliam, H.R. (1981). Learning to forage optimally. *In:* A.C. Kamil and T.D. Sargent (ed.) Foraging Behavior: Ecological, Ethological and Psychological approaches. Garland STPM Press, New York.

Sisodia, S and Singh, B. N. (2005). Behaviour genetics of *Drosophila*: Non-sexual behaviour. J. Genet. 84:195-216

APPENDIX A: SAS output for WGA of predatory mite *P. persimilis*

WGA of Predatory mite

Obs	enzyme	gDNA	WGA
1	0.30	1.00	2832.50
2	0.30	1.00	1293.00
3	0.30	1.00	2182.30
4	0.30	1.00	1071.20
5	0.30	0.30	2893.10
6	0.30	0.30	1605.00
7	0.30	0.30	1940.00
8	0.30	0.30	1078.30
9	0.30	0.10	1544.60
10	0.30	0.10	791.00
11	0.30	0.10	1656.00
12	0.30	0.10	717.40
13	0.30	0.03	2362.80
14	0.30	0.03	30.00
15	0.30	0.03	560.80
16	0.30	0.03	864.00
17	0.30	0.01	1847.70
18	0.30	0.01	699.00
19	0.30	0.01	903.50
20	0.30	0.01	100.80
21	0.10	1.00	2857.20
22	0.10	1.00	1665.50
23	0.10	1.00	1854.00
24	0.10	1.00	862.69
25	0.10	0.30	2966.40
26	0.10	0.30	1496.70
27	0.10	0.30	1725.00
28	0.10	0.30	761.80
29	0.10	0.10	1903.90
30	0.10	0.10	669.20
31	0.10	0.10	1755.00
32	0.10	0.10	0.70
33	0.10	0.03	2172.00
34	0.10	0.03	30.40
35	0.10	0.03	80.00
36	0.10	0.03	9.00
37	0.10	0.01	923.30
38	0.10	0.01	549.10
39	0.10	0.01	2.00
40	0.10	0.01	228.90
41	0.03	1.00	1884.00
42	0.03	1.00	2443.50
43	0.03	1.00	1308.00
44	0.03	1.00	741.40
45	0.03	0.30	3443.10
46	0.03	0.30	950.50
47	0.03	0.30	1368.00
48	0.03	0.30	903.30
49	0.03	0.10	759.00
50	0.03	0.10	210.50
51	0.03	0.10	1278.00
52	0.03	0.10	141.7
53	0.03	0.03	1705.3
54	0.03	0.03	2.3
55	0.03	0.03	69.0
56	0.03	0.03	10.9
57	0.03	0.01	0.1
58	0.03	0.01	467.5
59	0.03	0.01	0.0
60	0.03	0.01	0.5

WGA of Predatory mite
The GLM Procedure

Class Level Information

Class	Levels	Values
enzyme	3	0.03 0.1 0.3
gDNA	5	0.01 0.03 0.1 0.3 1

Number of Observations Read	60
Number of Observations Used	60

WGA of Predatory mite
The GLM Procedure

Dependent Variable: WGA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	19705440.38	3284240.06	5.87	<.0001
Error	53	29642239.80	559287.54		
Corrected Total	59	49347680.18			

R-Square	Coeff Var	Root MSE	WGA Mean
0.399318	66.80024	747.8553	1119.540

Source	DF	Type I SS	Mean Square	F Value	Pr > F
enzyme	2	2157046.80	1078523.40	1.93	0.1554
gDNA	4	17548393.58	4387098.40	7.84	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
enzyme	2	2157046.80	1078523.40	1.93	0.1554
gDNA	4	17548393.58	4387098.40	7.84	<.0001

The GLM Procedure

t Tests (LSD) for WGA

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	53
Error Mean Square	559287.5
Critical Value of t	2.00575
Least Significant Difference	474.34

Means with the same letter are not significantly different.

t Grouping	Mean	N	enzyme
A	1348.7	20	0.3
A			
A	1125.6	20	0.1
A			
A	884.3	20	0.03

The GLM Procedure

t Tests (LSD) for WGA

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	53
Error Mean Square	559287.5
Critical Value of t	2.00575
Least Significant Difference	612.38

Means with the same letter are not significantly different.

t Grouping	Mean	N	gDNA
A	1760.9	12	0.3
A			
A	1749.6	12	1
B	952.3	12	0.1
B			
B	658.0	12	0.03
B			
B	476.9	12	0.01

APPENDIX B: SAS output for consumption and oviposition behavior

Obs	trt	cons	ovi
1	1	21	2
2	1	32	4
3	1	16	3
4	1	31	5
5	1	11	2
6	1	7	2
7	1	32	6
8	1	26	3
9	1	20	2
10	1	29	3
11	2	11	2
12	2	31	3
13	2	27	3
14	2	19	5
15	2	6	4
16	2	33	5
17	2	29	3
18	2	18	3
19	2	16	4
20	3	19	3
21	3	27	5
22	3	32	4
23	3	19	2
24	3	28	5
25	3	17	2
26	3	13	2
27	3	9	2
28	4	33	6
29	4	18	3
30	4	29	5
31	4	32	5
32	4	12	2
33	4	37	4
34	4	17	3

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of Observations Read	34
Number of Observations Used	34

Consumption oviposiiton Behavior

The GLM Procedure

Dependent Variable: **cons**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	107.514472	35.838157	0.44	0.7240
Error	30	2427.103175	80.903439		
Corrected Total	33	2534.617647			

R-Square	Coeff Var	Root MSE	cons Mean
0.042418	40.39862	8.994634	22.26471

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	107.5144725	35.8381575	0.44	0.7240

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	107.5144725	35.8381575	0.44	0.7240

Consumption oviposiiton Behavior

The GLM Procedure

Dependent Variable: **ovi**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3.68513072	1.22837691	0.73	0.5440
Error	30	50.69722222	1.68990741		
Corrected Total	33	54.38235294			

R-Square	Coeff Var	Root MSE	ovi Mean
0.067763	37.77674	1.299964	3.441176

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	3.68513072	1.22837691	0.73	0.5440

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	3.68513072	1.22837691	0.73	0.5440

APPENDIX C: SAS output for dispersal behavior

Obs	TRT	DISH	TIME	D
1	1	1	1	0
2	1	1	2	3
3	1	1	3	0
4	1	1	4	2
5	1	1	5	0
6	1	1	6	0
7	1	1	7	0
8	1	1	8	0
9	2	1	1	0
10	2	1	2	1
11	2	1	3	0
12	2	1	4	1
13	2	1	5	0
14	2	1	6	0
15	2	1	7	1
16	2	1	8	1
17	3	1	1	0
18	3	1	2	0
19	3	1	3	0
20	3	1	4	1
21	3	1	5	1
22	3	1	6	0
23	3	1	7	1
24	3	1	8	0
25	4	1	1	0
26	4	1	2	0
27	4	1	3	0
28	4	1	4	2
29	4	1	5	0
30	4	1	6	1
31	4	1	7	0
32	4	1	8	2
33	1	2	1	0
34	1	2	2	2
35	1	2	3	1
36	1	2	4	0
37	1	2	5	1
38	1	2	6	0
39	1	2	7	0
40	1	2	8	0
41	2	2	1	0
42	2	2	2	0
43	2	2	3	0
44	2	2	4	0
45	2	2	5	2
46	2	2	6	0
47	2	2	7	1
48	2	2	8	0
49	3	2	1	0
50	3	2	2	1
51	3	2	3	0
52	3	2	4	1
53	3	2	5	1
54	3	2	6	1
55	3	2	7	0
56	3	2	8	0
57	4	2	1	0
58	4	2	2	1
59	4	2	3	0
60	4	2	4	1

61	4	2	5	0
62	4	2	6	1
63	4	2	7	2
64	4	2	8	0
65	1	3	1	0
66	1	3	2	3
67	1	3	3	0
68	1	3	4	2
69	1	3	5	0
70	1	3	6	0
71	1	3	7	0
72	1	3	8	0
73	2	3	1	0
74	2	3	2	1
75	2	3	3	0
76	2	3	4	0
77	2	3	5	0
78	2	3	6	0
79	2	3	7	1
80	2	3	8	0
81	3	3	1	0
82	3	3	2	0
83	3	3	3	0
84	3	3	4	1
85	3	3	5	0
86	3	3	6	0
87	3	3	7	0
88	3	3	8	1
89	4	3	1	0
90	4	3	2	0
91	4	3	3	0
92	4	3	4	2
93	4	3	5	0
94	4	3	6	0
95	4	3	7	1
96	4	3	8	1
97	1	4	1	0
98	1	4	2	2
99	1	4	3	1
100	1	4	4	1
101	1	4	5	0
102	1	4	6	1
103	1	4	7	0
104	1	4	8	0
105	2	4	1	0
106	2	4	2	0
107	2	4	3	0
108	2	4	4	2
109	2	4	5	0
110	2	4	6	0
111	2	4	7	0
112	2	4	8	1
113	3	4	1	0
114	3	4	2	0
115	3	4	3	0
116	3	4	4	1
117	3	4	5	0
118	3	4	6	0
119	3	4	7	1
120	3	4	8	1
121	4	4	1	0
122	4	4	2	1
123	4	4	3	0
124	4	4	4	0
125	4	4	5	2
126	4	4	6	1
127	4	4	7	0
128	4	4	8	0

REPEATED MEASURES EXPERIMENT
ASSUMING COMPOUND SYMMETRY

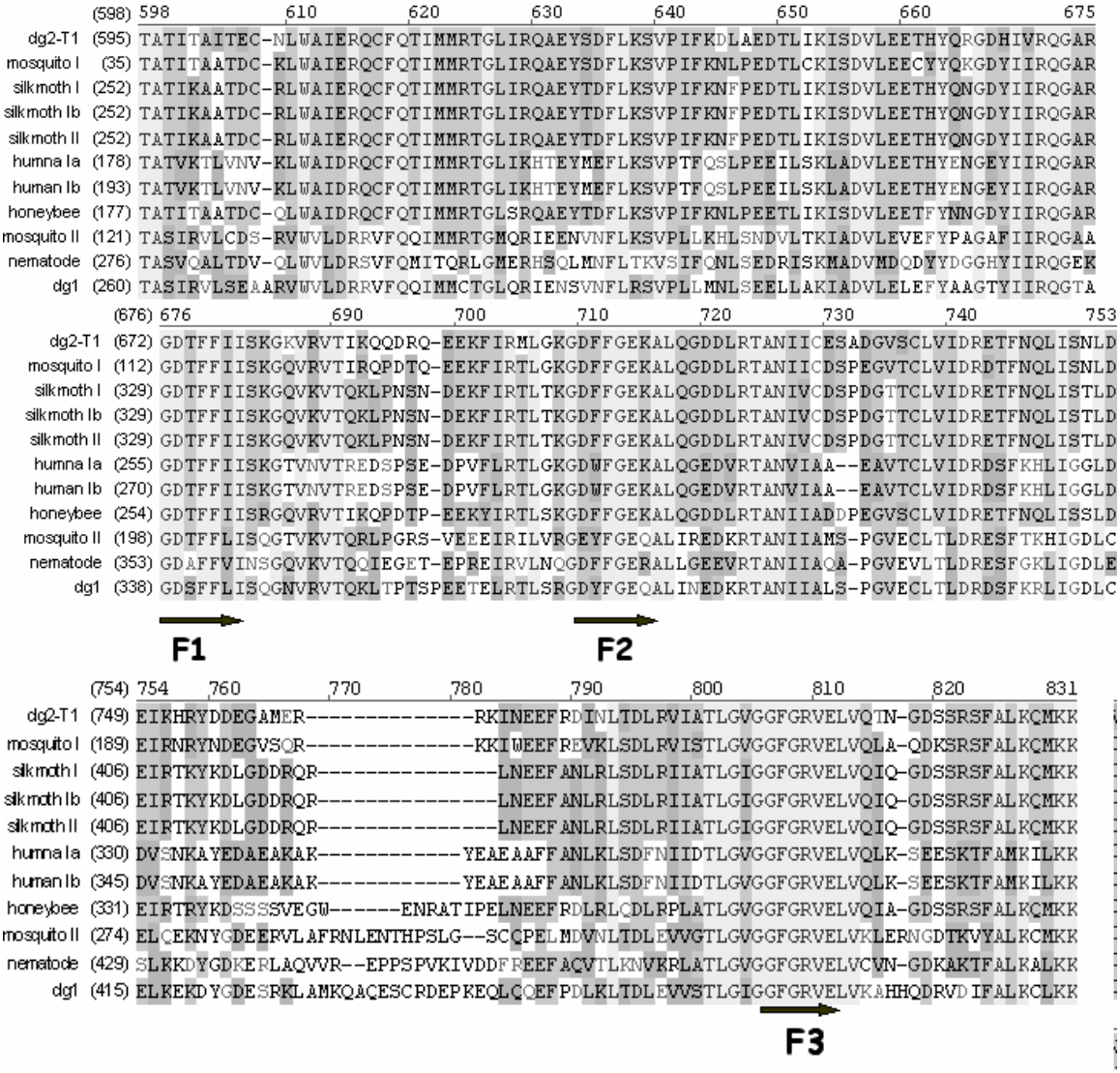
The GLM Procedure
Tests of Hypotheses for Mixed Model Analysis of Variance

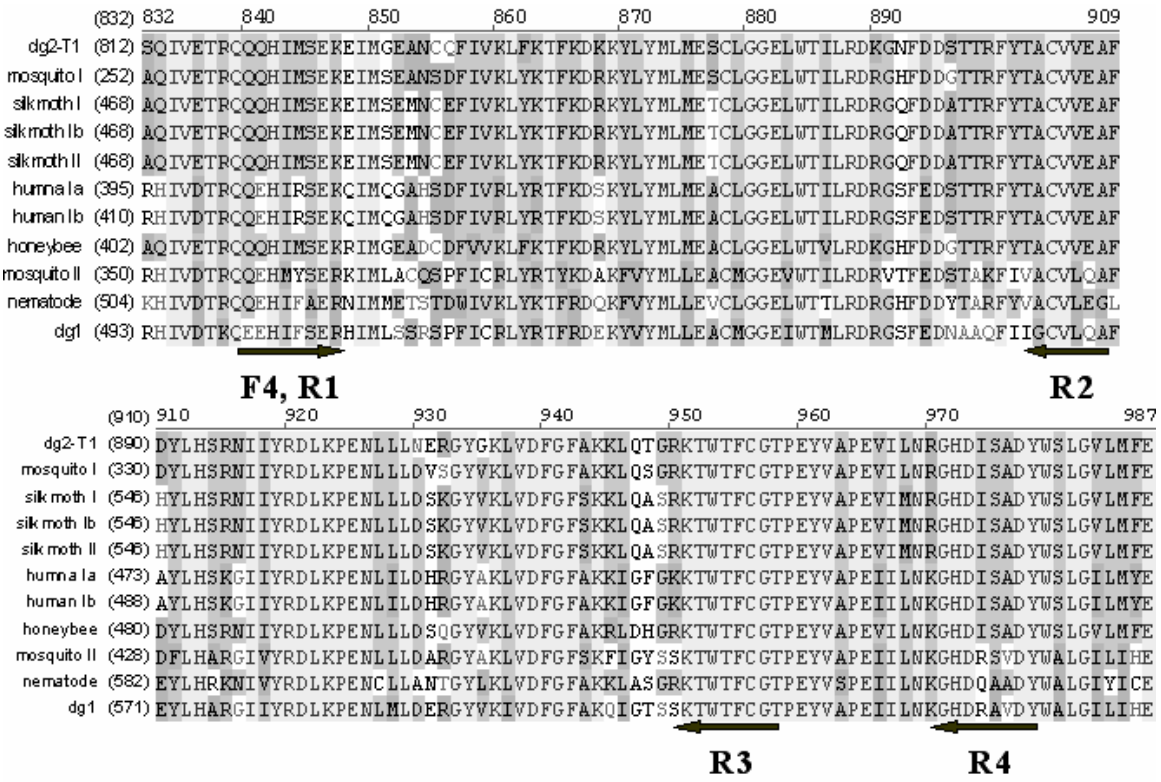
Dependent Variable: D

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
*	TRT	3	1.335937	0.445312	7.43	0.0045
	Error: MS(DISH(TRT))	12	0.718750	0.059896		

* This test assumes one or more other fixed effects are zero.

APPENDIX D: *for* gene alignment for designing degenerate primers. The alignment shows four forward primers and four reverse primers




(832) 832 840 850 860 870 880 890 909
dg2-T1 (812) SQIVETRCQQHIMSEKEIMGEANCQFIVKLFKTFKDKKYLYMLMESCLGGELWTILRDKGNFDDSTTRFYTACVVEAF
mosquito I (252) AQIVETRCQQHIMSEKEIMSEANSDFIVKLYKTFKDRKYLYMLMESCLGGELWTILRDRGHFDDGTTRFYTACVVEAF
silk moth I (468) AQIVETRCQQHIMSEKEIMSEMNCEFIVKLYKTFKDRKYLYMLMETCLGGELWTILRDRGQFDDATTRFYTACVVEAF
silk moth Ib (468) AQIVETRCQQHIMSEKEIMSEMNCEFIVKLYKTFKDRKYLYMLMETCLGGELWTILRDRGQFDDATTRFYTACVVEAF
silk moth II (468) AQIVETRCQQHIMSEKEIMSEMNCEFIVKLYKTFKDRKYLYMLMETCLGGELWTILRDRGQFDDATTRFYTACVVEAF
humna Ia (395) RHIVDTRCQEHIRSEKCIMCGAHSDFIVRLYRTFKDSKYLYMLMEACLGGELWTILRDRGSFEDSTTRFYTACVVEAF
human Ib (410) RHIVDTRCQEHIRSEKCIMCGAHSDFIVRLYRTFKDSKYLYMLMEACLGGELWTILRDRGSFEDSTTRFYTACVVEAF
honeybee (402) AQIVETRCQQHIMSEKRIMGEADCDFVVKLFKTFKDRKYLYMLMEACLGGELWTVLRDKGHFDDGTTRFYTACVVEAF
mosquito II (350) RHIVDTRCQEHMYSERKIMLACQSPFICRLYRTYKDAKFVYMLLEACMGGEVWTILRDRVTFEDSTAKFIVACVLQAF
nematode (504) KHIVDTRCQEHIFAERNIMMETSTDWIVKLYKTFRDQKFVYMLLEVCLGGELWTTLRDRGHFDDYTARFYVACVLEGL
dg1 (493) RHIVDTKCEEHIFSERHIMLSSRSPFICRLYRTFRDEKYVYMLLEACMGGEIWTMLRDRGSFEDNAAQFIIGCVLQAF
F4, R1
R2
(910) 910 920 930 940 950 960 970 987
dg2-T1 (890) DYLHSRNIIYRDLKPENLLLNERGYGKLVDFGFAKKLQTGRKTWTFCGTPEYVAPEVILNRGHDISADYWSLGVLMFE
mosquito I (330) DYLHSRNIIYRDLKPENLLLDVSGYVKLVDFGFAKKLQSGRKTWTFCGTPEYVAPEVILNRGHDISADYWSLGVLMFE
silk moth I (546) HYLHSRNIIYRDLKPENLLLDSKGYVKLVDFGFSKKLQASRKTWTFCGTPEYVAPEVIMNRGHDISADYWSLGVLMFE
silk moth Ib (546) HYLHSRNIIYRDLKPENLLLDSKGYVKLVDFGFSKKLQASRKTWTFCGTPEYVAPEVIMNRGHDISADYWSLGVLMFE
silk moth II (546) HYLHSRNIIYRDLKPENLLLDSKGYVKLVDFGFSKKLQASRKTWTFCGTPEYVAPEVIMNRGHDISADYWSLGVLMFE
humna Ia (473) AYLHSKGIIYRDLKPENLILDHRGYAKLVDFGFAKKIGFGKKTWTFCGTPEYVAPEIILNKGHDISADYWSLGILMYE
human Ib (488) AYLHSKGIIYRDLKPENLILDHRGYAKLVDFGFAKKIGFGKKTWTFCGTPEYVAPEIILNKGHDISADYWSLGILMYE
honeybee (480) DYLHSRNIIYRDLKPENLLLDSQGYVKLVDFGFAKRLDHGRKTWTFCGTPEYVAPEVILNKGHDISADYWSLGVLMFE
mosquito II (428) DFLHARGIVYRDLKPENLLLDARGYAKLVDFGFSKFIGYSSKTWTFCGTPEYVAPEIILNKGHDRSVDYWALGILIHE
nematode (582) EYLHRKNIVYRDLKPENCLLANTGYLKLVDFGFAKKLASGRKTWTFCGTPEYVSPEIILNKGHDQAADYWALGIYICE
dg1 (571) EYLHARGIIYRDLKPENLMLDERGYVKIVDFGFAKQIGTSSKTWTFCGTPEYVAPEIILNKGHDRAVDYWALGILIHE
R3
R4