# Chapter 2
# Improvement of Entomopathogenic Nematodes: A Genetic Approach

**Itamar Glazer**

## 2.1 Introduction

Domestication and improvement of crop plants and animals have been part of agriculture for thousands of years, and many agricultural systems are therefore artificial. Genetic manipulation of other beneficial arthropods, such as silkworms and honeybees, has been conducted for hundreds of years (Hoy, 1990; Yokoyama, 1973). As in crop breeding, four potential genetic–manipulation strategies exist: artificial selection, hybridization (use of heterosis), mutation, and recombinant DNA techniques.

Genetic improvement programs (GIPs) have also provided innovative methods for controlling insect pests (Hoy, 1985c, 1986). Beneficial arthropods have been selected for climate tolerance (White, DeBach, & Garber, 1970; Wilkes, 1942), host–finding ability, host preference (Allen, 1954; Box, 1956), improved sex ratio (Simmonds, 1947; Wilkes, 1947), increased fecundity (Ram & Sharma, 1977; Wilkes, 1947), and resistance to insecticides (Havron, Kenan, & Rosen, 1991; Havron, Rosen, Prag, & Rossler, 1991; Hoy, 1984; Hoy & Cave, 1991; Hoy, Conley, & Robinson, 1988; Pielou & Glasser, 1952; Roush & Hoy, 1981).

Hoy (1985a, 1990) suggested a number of steps that need to be taken for the genetic improvement of biological control agents of arthropods. These include:

1. Identification of the factors limiting the efficacy of the natural enemy and more specifically, identification of the traits that need improving.
2. Genetic variability must be available for artificial selection.

I. Glazer (✉)
Division of Nematology, Department of Entomology, Institute of Plant Protection, Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel
e-mail: glazerit@vulcani.agri.gov.il

© Springer International Publishing Switzerland 2015

R. Campos-Herrera (ed.), *Nematode Pathogenesis of Insects and Other Pests*,
Sustainability in Plant and Crop Protection 1, DOI 10.1007/978-3-319-18266-7_2

3. Genetic improvement by selection, hybridization, mutagenesis or recombinant DNA methods.
4. Evaluation of genetically improved strains in laboratory, greenhouse and field for efficacy, fitness and stability.
5. Cost–benefit evaluation: one must assume that the cost of the project will be justified by the benefits achieved.

For example *Metaseiulus occidentalis* (Nesbitt) (Mesostigmata; Phytoseiida) is an effective predator of spider mites in deciduous orchards and vineyards in western North America (Hoy, 1985b). It acquired resistance to organophosphorus (OP) insecticides through natural selection in apple orchards in Washington State, and this resistance allowed the predator to survive in orchards even though an OP insecticide azinphos–methyl was applied to control codling moth (Hoyt, 1969). A GIP for *M. occidentalis* involving selection for resistance to carbaryl and permethrin was initiated, and multiresistant strains of *M. occidentalis* were obtained through laboratory crosses and additional selections. The laboratory–selected strains were then tested in small–plot trials for 2 years to determine whether they could become established in orchards or vineyards, survive the relevant pesticide applications in the field, spread, multiply, overwinter, and control the spider mites (Hoy). The small–plot trials were then followed by 3 years of research into how to implement the predators in an integrated mite–management program in almonds (Hoy). The economic analysis suggested that almond growers adopting this program would save $60–$110 per hectare. Thus, genetic improvement of *M. occidentalis* has been shown to be efficacious and cost–effective.

Unlike the long history and vast research on the use of beneficial insects for biological control, the use of entomopathogenic nematodes (EPNs) is only in its third decade; consequently, research and development of GIPs for EPNs is in its infancy. In the present chapter, the need for, and advances in the establishment of genetic approaches for trait improvement in steinernematid and heterorhabditid EPNs will be reviewed, using the scheme proposed by Hoy (1990) as a benchmark.

As the use of EPNs for biological control of insect pests becomes practical and commercial, due to improvements in production methods (Grewal, Ehlers, & Shapiro-Ilan, 2005; Shapiro-Ilan, Han, & Dolinksi, 2012), the use of powerful genetic tools to improve their performance has been strongly advocated (see reviews by Burnell & Dowds, 1996; Gaugler, 1987; Fodor, Vecseri, & Farkas, 1990; Segal & Glazer, 1998, 2000). In this chapter, I describe the reported GIPs of EPNs, the experience gained from these attempts, and future possibilities.

## 2.2 Identification of Traits for Improvement by Genetic Means

To consider a GIP, one must identify the traits that need to be improved. In general, two directions for EPN improvement have been suggested. The first is enhancement of EPN field efficacy by improving their infectivity to certain insects

or their ability to overcome environmental factors affecting their performance and biocontrol consistency. The second is enhancement of their commercial suitability, i.e., increased production efficiency and consistency as well as shelf–life stability. These goals need to be "translated" into defined procedures that can be used in a GIP. Knowledge of the genetic, molecular and physiological architecture of particular traits related to the improvement goal must be established.

### *2.2.1 Infectivity*

It has been established that nematode infectivity to different insects or developmental stages of a host varies considerably (Caroli, Glazer, & Gaugler, 1996; Ricci, Glazer, & Gaugler, 1996). Moreover, the process of EPN infectivity and virulence is quite complex (see Chap. 1 for more details). Successful infection and establishment of nematodes in the insect rely on their ability to locate and invade the insect, overcome its immune system and successfully release their symbiotic bacteria. Each of these steps, and possibly some secondary ones, should be considered for improvement and must be studied to determine their effect on the overall infectivity process, before a GIP is initiated. Little is known about the EPN's mechanism of infection or the genes involved in this process. Hao, Montiela, Abubuckerb, Mitrevab, and Simoes (2010), in a study of parasitic mechanisms exhibited by *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), generated a cDNA library of the induced *S. carpocapsae* parasitic phase. Comparative analysis identified 377 homologs in *Caenorhabditis elegans* Maupas (Rhabditida (Rhabditoidea)), 431 in *Caenorhabditis briggsae* Osche (Rhabditida; Rhabditoidea), and 75 in other nematodes. Classification of the predicted proteins revealed involvement in diverse cellular, metabolic and extracellular functions: 119 clusters were predicted to encode putatively secreted proteins such as proteases, protease inhibitors, lectins, saposin–like proteins, acetylcholinesterase, antioxidants, and heat–shock proteins, which might interact with the host. This dataset provided the basis for genomic studies toward a better understanding of the events that occur in the parasitic process of this EPN, including invasion of the insect hemocoel, adaptation to the insect's innate immunity and stress responses, and production of virulence factors. The identification of key genes in the parasitic process will provide useful tools for the improvement of *S. carpocapsae* infectivity.

### *2.2.2 Survival and Persistence*

Once EPNs are released into the field environment, they encounter numerous factors—including physical, chemical and biological components—that affect their survival and activity as biological control agents (see Chaps. 3, 4 and 5 for more details). It is assumed that during the course of evolution, EPNs, like other terrestrial

organisms, adopted unique survival mechanisms to resist environmental extremes. Several researchers noted that enhancement of their ability to survive the environmental factors will increased their efficacy by enabling more nematodes to persist and infect longer periods in the field (Gaugler, 1987; Segal & Glazer, 1998, 2000). Thus, genetic improvement has been suggested as means to achieve such enhancement. However, as of yet, there is no direct evidence for this notion with EPNs.

One of the major obstacles to using EPNs in commercial pest control is their limited shelf life (Strauch, Oestergaard, Hollmer, & Ehlers, 2004). The longevity of the nematode infective juveniles (IJs) can be prolonged by inducing a quiescent state in which their metabolic activity is much reduced. In general, this is done by storage at low temperature (Grewal et al., 2005). However, EPNs are often exposed to higher temperatures during transportation (Mukuka, Strauch, & Ehlers, 2010b), which reduces their viability. Selective breeding for enhancement of desiccation and heat tolerance as a means of prolonging their shelf life, and their capacity for storage and transportation has been suggested.

Nematodes, like bacteria, fungi, and plants, can survive unfavorable environmental conditions in a quiescent state, which considerably prolongs their life span and enables them to withstand the rigors of a fluctuating regime (Barrett, 1991; Watanabe, 2006). Unfavorable environmental conditions include lack of water, extreme temperatures, lack of oxygen, and osmotic stress; the types of quiescence induced in organisms by these conditions are termed anhydrobiosis, thermobiosis/cryobiosis, anoxybiosis and osmobiosis, respectively (Barrett). As the importance of EPNs as biological control agents rises, substantial information regarding their survival mechanisms is being published. Two traits in particular are considered to be most important: desiccation and heat tolerance (Glazer, 2002).

Nematodes are aquatic organisms that need to have a film of water surrounding their body to move (Norton, 1978). Dry conditions adversely affect nematode motility and survival. Some nematode species have adopted anhydrobiosis as a means of surviving prolonged dry periods. This quiescent state in usually reached following a slow rate of water loss (Crowe & Madin, 1975). EPNs can persist for 2–3 weeks in dry soil (Kaya, 1990; Kung & Gaugler, 1990). Most studies investigating the steinernematid nematodes' ability to survive desiccation have focused on *S. carpocapsae* (e.g., Glazer, 1992; Ishibashi, Tojo, & Hatate, 1987; Simons & Poinar, 1973; Womersley, 1990). The general finding has been that various strains of *S. carpocapsae* can survive for appreciable lengths of time under slow drying conditions. In addition, all populations of *S. carpocapsae* survived desiccation better than *Steinernema glaseri* (Steiner) (Rhabditida: Steinernematidae) (Kung, Gaugler, & Kaya, 1990) and *Steinernema riobrave* (Cabanillas, Poinar and Raulston) (Rhabditida: Steinernematidae) (Baur, Kaya, & Thurston, 1995). Solomon, Solomon, Paperna, and Glazer (2000) studied the desiccation tolerance of populations IS–6, IS–15 and SF of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae). Population IS–6 isolated from the desert region of Israel exhibited the highest survival ability, followed by population IS–15 isolated from northern Israel. The poorest tolerance to desiccation was exhibited by population SF, which was isolated in Germany.

As indicated earlier, fewer studies have been devoted to determining the desiccation tolerance of heterorhabditids. Previous studies have indicated that nematodes belonging to this genus are poor anhydrobionts (Menti, Wright, & Perry, 1997; O'Leary, Stack, Chubb, & Burnell, 1998; Surrey & Wharton, 1995). Liu and Glazer (2000) found wide diversity in desiccation tolerance of heterorhabditid populations from Israel. Furthermore, as a basis for genetic selection, Mukuka, Strauch, Al Zainab, and Ehlers (2010) screened the desiccation tolerance of 43 populations of *Heterorhabditis* spp. and 18 hybrid/inbred strains of *H. bacteriophora* showing significant interspecific variation between nematode populations and species. High variation was found among the different populations, which is an essential prerequisite for the initiation of a GIP (Segal & Glazer, 2000).

The genetic and biochemical mechanisms involved in the induction of anhydrobiosis are not fully understood. One biochemical change that has been reported in anhydrobiotic nematodes is the accumulation of polyols and sugars, in many cases trehalose, which are believed to protect the biological membranes and intracellular proteins during dehydration (e.g., Watanabe, 2006; Womersley, 1990). Solomon, Paperna, and Glazer (1999) showed an increase in trehalose levels in *S. feltiae* IJs exposed to slow dehydration conditions; following rehydration, trehalose concentrations decreased by 50 % within 24 h (Solomon et al., 1999). The synthesis and accumulation of proteins during the desiccation process have been characterized in bacteria, fungi, yeast and plant seeds (Close, 1996; Dure, 1993), but little is known about these aspects in nematodes. In this regard, Chen, Gallop, and Glazer (2005) and Chen et al. (2005) identified novel proteins in osmotically stressed IJs of *S. feltiae* using two–dimensional electrophoresis. Ten novel protein spots and ten upregulated protein spots were detected in the osmotically desiccated IJs. Mass spectrometry analysis of seven significant spots indicated that osmotic stress in desiccated IJs is associated with the induction of actin, proteasome regulatory particle (ATPase–like), GroEL chaperonin, and GroES co–chaperonin.

Gene functions and molecular mechanisms involved in desiccation tolerance in EPNs are just at early stage of investigation. Gal, Solomon, Glazer, and Koltai (2001) identified novel genes of *S. feltiae* population IS–6 that exhibit changes in transcript level upon dehydration. These included the gene encoding glycogen synthase (*Sf–gsy–1*), the rate–limiting enzyme in the synthesis of glycogen, the latter likely playing a role in desiccation survival. Solomon et al. (2000) identified a heat–stable, water stress–related protein with a molecular mass of 47 kDa (designated *desc*47) in *S. feltiae* population IS–6. It was characterized as a late embryogenesis abundant (LEA) homolog protein belonging to the LEA group 3–like proteins. The LEA proteins are a diverse group of water stress–related proteins that are expressed in maturing seeds and in water deficit–stressed vegetative tissues of higher plants (Close, 1996), as well as in nematodes (Burnell & Tunnacliffe, 2011; Tyson, Reardon, Browne, & Burnell, 2007).

Using subtraction hybridization and differential display, several classes of *S. feltiae* genes that are induced in IJs in response to desiccation stress were isolated (Gal, Glazer, & Koltai, 2003). These included transcriptional regulators of metabolic enzymes that are involved in the production of osmoregulants and proteinaceous

stress protectants. Among the identified stress–related genes were those encoding *S. feltiae* NAP–1 (nucleosome–assembly protein; *Sf–nap–1*) and CK2 (casein kinase 2; *Sf–ck2*) (Gal et al., 2003; Gal, Glazer, & Koltai, 2005; Gal, Glazer, Sherman, & Koltai, 2005). Interaction between the proteins *Sf*–NAP–1 and *Sf*–CK2was indicated using the yeast two–hybrid system.

A functional role for a LEA protein in *C. elegans* (Ce–LEA–1) in the response to stress conditions was demonstrated (Gal, Glazer, & Koltai, 2004). Group 3 LEA proteins, which are prominent components of the stress response in various organisms (Wise & Tunnacliffe, 2004), are thought to be mainly involved in counteracting the irreversible damage caused by the increased ionic strength that develops in the cytosol during desiccation, perhaps through the binding of both anions and cations to the helical region of the protein (Ingram & Bartels, 1996). The steady–state level of *Ce–lea–1* mRNA increased upon dehydration of *C. elegans* dauer larvae. Partial silencing of this mRNA level steady–state, reduced dauer larvae survival under desiccation, osmotic–stress and heat–stress conditions (Gal et al., 2004). Therefore, it was suggested that Ce–LEA–1 is a critical component of the nematodes' strategy for tolerating the water losses associated with dehydration, osmotic and heat stresses (Gal et al.). The common requirement for Ce–LEA–1 for survival during the examined stresses might support the concept of a molecular mechanism in nematodes that is common to several stress responses, making it a key candidate genetic manipulation toward enhancement of stress tolerance in EPNs.

Somvanshi, Koltai, and Glazer (2008) investigated gene expression in nematodes that were tolerant or susceptible to desiccation stress to determine whether enhanced tolerance in these populations results from a 'gene–expression response' to desiccation or if, for enhanced tolerance, no such response is needed, perhaps due to a state of constant 'readiness'. The expressions of four stress representative genes —*aldehyde dehydrogenase*, *nucleosome assembly protein 1*, *glutathione peroxidase* and *heat–shock protein 40* — were characterized during desiccation stress in five EPN species with differing levels of stress tolerance: *S. feltiae* population IS–6, *S. feltiae* Carmiel population, *S. carpocapsae* Mexican population, *S. riobrave*, and *H. bacteriophora* population TTO1. After 24 h of desiccation, an inverse relationship between the expression of the studied genes and phenotypic desiccation–tolerance capability in the nematodes was observed. *H. bacteriophora* TTO1 was most susceptible to desiccation but showed the highest expression of all studied genes under desiccation. *S. carpocapsae* Mexican population and *S. riobrave* showed the lowest expression of these genes but were most tolerant to desiccation. This study showed no induction of gene expression in stress–tolerant nematodes, whereas the stress–susceptible nematodes responded to stress by induced expression of these genes. Since the different levels of gene expression were found to be related to the different stress–tolerance capabilities of the nematodes, such gene–expression ratios can potentially be used as markers of desiccation tolerance in EPNs. Furthermore, these results imply that molecular tools for the reduction of gene expression, such as RNA interference (RNAi) or genome editing (see further on), may be useful for increasing EPNs' tolerance to stress.

## 2.3 Genetic Improvement of Entomopathogenic Nematodes

As the advances in production methods rendered the use of EPNs for the biological control of insect pests more practical and commercially feasible (Grewal et al., 2005; Shapiro-Ilan et al., 2012), several attempts were made to improve their performance by genetic means. In this section, I list the reported GIPs of EPNs (summarized in Table 2.1), the experience gained from these attempts, and future possibilities.

Prior to any selection for genetic improvement, it is essential to determine the genetic variation of the particular trait (expressed in terms of 'heritability', $h^2$) that must be present in the population. Glazer, Gaugler and Segal (1991) assessed the genotypic variation among IJs of *H. bacteriophora* population HP88 under heat, desiccation and ultraviolet light by comparing the performance of inbred lines of this nematode in laboratory assays. Considerable variation in all three traits was detected among the different inbred lines. The heritability values for heat and ultraviolet tolerance were high ($h^2 = 0.98$ and 0.66, respectively), indicating that selection should be an efficient way of improving these traits in the population. The results for desiccation tolerance varied considerably within each line. The heritability value was low ($h^2 = 0.11$), indicating that the results were influenced mainly by environmental variation and suggesting that selective breeding for higher desiccation tolerance would be inefficient. Improvement through the induction of mutations might be a better approach.

To improve the production of *H. bacteriophora* in liquid culture, Johnigk, Hollmer, Strauch, Wyss, and Ehlers (2002), determined the heritability of the disposition to recover in 30 homozygous inbred lines which were established by inbreeding over seven generations. The $h^2$ values of IJ recovery, as well as final yield, were determined in liquid culture, because the proportion of IJs that recover from the infective stage to the developmental reproductive parasitic stage varies considerably in liquid culture, thus affecting the consistency of production. The calculated heritability for IJ recovery was low ($h^2 = 0.38$). No significant genetic variability could be detected for this trait. In contrast, high heritability ($h^2 = 0.90$) was found for the total number of IJs produced in the liquid medium.

Additional heritability values for different traits which have been the subject of genetic improvement in EPNs are given below and listed in Table 2.1. In the future, additional information and tools for genetic improvement are needed. That includes development of genetic markers as well as identification of specific genes and genetically define traits that can be transferred between designated populations.

**Table 2.1** List of genetic improvement programs for entomopathogenic nematodes

| Nematode species | Improvement goal | Approach | Heritability ($h^2$) | Methodology | Results | Fitness change | Trait stability | Comments | References |
|---|---|---|---|---|---|---|---|---|---|
| *Steinernema feltiae* | Host-finding ability | Selection | 0.50 | 13 rounds in host-finding bioassay | 20- to 27-fold increase in host finding | Gain in host penetration and reproductive potential, loss in some storage stability | Gradual decrease | Used high-variation foundation strain | Gaugler and Campbelll (1989) Gaugler, Campbell, and McGuire (1990) |
| *S. feltiae* | Efficacy against *L. solani* | Selection | ND[a] | 33 rounds in host-infection bioassay | 4-fold increase in efficacy | NT[b] | NT | In field trial, showed better efficacy than standard EPNs | Tomalak, (1989, 1994a) Grewal, Tomalak, Keil, and Gaugler (1993) |
| *S. feltiae* | Desiccation (rapid and slow) tolerance | Selection | ND | 20 selection rounds | Significant increase in survival (>85 %) | No change | Gradual decrease | Heterogeneous foundation population | Salame, Glazer, Chubinishvilli, and Chkhubianishvili (2010) |
| *S. feltiae* | Desiccation tolerance | Gene transformation | ND | Microinjection of trehalose-6-phosphate synthase | Enhancement of osmotic tolerance (in adults) | NT | NT | | Vellai et al. (1999) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *S. carpocapsae* | Desiccation and Heat tolerance + Virulence | Hybridization | ND | Crosses between strains, 'Italian' and DD-136 | Increase in all trait in 2 of 3 modified strains | NT | NT | | Shapiro-Ilan et al. (2005) |
| *S. carpocapsae* | Enhanced dispersal | Selection | 0.60 | Capturing the fastest and farthest reaching IJs emanating from an infected *G. mellonella* cadaver, in soil. | 21–37 fold increase in the percent IJs dispersing from the source cadaver | Reduced reproduction capacity and nictation ability, a | | The selected lines comprised more males (72 %) than the foundation population (44 %) | Bal, Michel, and Grewal (2014) |
| *Heterorhabditis bacteriophora* | Heat tolerance | Hybridization | 0.98 | Crosses between strains IS-5 and HP88 | Increase in heat tolerance | Loss in low temperature storage | NT | Mutants were used as genetic markers | Shapiro-Ilan, Glazer, and Segal (1997), Koltai, Glazer, and Segal (1994) |
| *H. bacteriophora* | Heat tolerance | Selection | 0.68 | 4 selection rounds | Increase from 38.5 to 39.2 °C | NT | NT | | Ehlers, Oestergaard, Hollmer, Wingen, and Strauch (2005) |
| *H. bacteriophora* | Cold tolerance | Selection | 0.38 | 4 selection rounds | Reduction from 7.3 to 6.1 °C | NT | NT | | Ehlers et al. (2005) |

(continued)

**Table 2.1** (continued)

| Nematode species | Improvement goal | Approach | Heritability ($h^2$) | Methodology | Results | Fitness change | Trait stability | Comments | References |
|---|---|---|---|---|---|---|---|---|---|
| *H. bacteriophora* | Desiccation tolerance in osmotic solution | Selection | ND | 8 selection rounds | Increased tolerance (from 0.89 to 0.81 $a_w$) | NT | NT | Used inbred and hybrid lines | Strauch, Oestergaard, Hollmer, and Ehlers (2004) |
| *H. bacteriophora* | Heat tolerance | Selection | 0.68 | 11 selection rounds | Increase of 3.0-5.5 °C | No change | NT | | Mukuka, Strauch, and Ehlers et al. (2010a) |
| *H. bacteriophora* | Desiccation tolerance in osmotic solution | Selection | ND | 6 selection rounds | Reduced $a_w$ value | No change | NT | | Mukuka, Strauch and Ehlers et al. (2010b) |
| *H. bacteriophora* | Nematicidal resistance to: | Selection | | 11 selection rounds | Fast increase | | | Cross-resistance was detected | Glazer, Salame, and Segal (1997) |
| | Enamiphos | | 0.31 | | 9-fold | No change | Decreased | | |
| | Xamyl | | 0.71 | | 70-fold | ” | Stable | | |
| | Vermectin | | 0.46 | | 8-fold | ” | Stable | | |
| *H. bacteriophora* | Heat tolerance | Gene transformation | ND | Microinjection of heat-shock protein gene *hsp16* | Enhancement of thermotolerance | No change | | | Hashmi, Hashmi, and Gaugler (1995, 1998) |

[a] *ND* not determined
[b] *NT* not tested

### 2.3.1 Enhancement of Infectivity

One of the first targets for improvement was the host–finding ability of EPNs. Gaugler and Campbell (1989, 1991) showed that sufficient phenotypical differences exist for host finding between geographical isolates of *S. feltiae* to expect a strong response to selection for this trait. Genetic variation was maximized by hybridizing 10 genetically diverse isolates to create a foundation population for selective breeding. Thirteen rounds of selection resulted in a 20- to 27-fold increase in host finding. Moreover, the proportion of IJs initiating positive chemotaxis increased from less than 33 % to more than 80 %. Nematodes failing to migrate out of the inoculation zone declined from 33 to 8 % after six rounds of selection. Relaxing the selection pressure produced a gradual decrease in host finding. This regression, coupled with the high realized heritability for enhanced host finding (0.64), suggested that wild–type populations take a passive approach to host finding.

The selected population for improved host finding (G–13) was compared to two wild–type populations—the 'All' population and the foundation population from which the G–13 population was derived—for changes in fitness (Gaugler et al., 1990). Acquisition of enhanced host–finding abilities did not appear to be correlated with a serious reduction in overall fitness. Selection did not affect pathogenicity, mobility, sex ratio or morphology. However, population G–13 did show a gain of fitness with regard to host penetration and reproductive potential, and a loss of fitness for storage stability.

Hiltpold, Baroni, Toepfer, Kuhlmann, Turlings (2010a) selected the EPN *Heterorhabditis bacteriophora* Poinar (Rhabditia; Heterorhabditidae) higher responsiveness towards (*E*)–β–caryophyllene (EβC), a sesquiterpene that is emitted by maize roots in response to feeding damage by the western corn rootworm (WCR). EβC is normally only weakly attractive to *H. bacteriophora*, which is one of the most infectious nematodes against WCR. By selecting *H. bacteriophora* to move more readily along a EβC gradient they obtained a population that was almost twice more efficient in controlling WCR population in fields planted with an EβC–producing maize variety. Tomalak (1994a) selected *S. feltiae* population PL for improved efficacy against *Lycoriella solani* (Winn.) (Diptera: Sciaridae) by repeated passage through this fly's larvae *in situ* in compost. There was a four-fold improvement in infectivity of the selected population (ScP) to *L. solani* in laboratory tests after 33 rounds of selection. The control potential of the genetically selected population ScP was further evaluated for the management of *Lycoriella mali* (Fitch), (Diptera: Sciaridae) (Grewal et al., 1993). Trials were conducted at two commercial mushroom farms with high and low levels of fly infestation. The efficacy of population ScP was compared with that of *S. feltiae* population SN and the chitin–synthesis inhibitor diflubenzuron. At low densities of *L. mali*, the two populations did not differ in efficacy, both causing 85–94 % reduction in fly populations. At high fly densities, and a mixed infestation with the phorid fly, *Megaselia halterata* (Wood) (Diptera: Phoridae), population ScP caused 56–83 % reduction in *L. mali* populations, whereas population SN caused 51–73 % reduction. Population ScP

persisted longer than population SN. Tomalak (1994b) also reported on the genetic improvement of *S. feltiae* for control of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). In this study, partially inbred lines and recombinant congenic populations were used. They were selected for high infectivity and small body diameter, showing a substantial increase (>40 %) in infectivity and efficacy.

### *2.3.2 Improvement of Survival*

Nematode tolerance and activity under extreme environmental conditions can limit the shelf life, quality and field performance of nematode–based products. As noted above, enhancement of EPN survival is considered a high priority for genetic improvement.

Shapiro-Ilan, Glazer, and Segal (1997) demonstrated the ability to transfer heat tolerance between two populations of *H. bacteriophora*. Transfer of this trait was accomplished by mating the heat–tolerant population IS–5 (Glazer, Kozodoi, Hashmi, & Gaugler, 1996) with the laboratory population HP88. The hybrid nature of the progeny was confirmed with a mutant of the HP88 population (*Hp–dpy–2*) as marker (Koltai et al., 1994) and by back–crossing. Progeny from the cross were screened for heat tolerance by measuring survival after 2 h exposure to 40 °C. After six passages through last–instar larvae of the wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae), survival of the hybrid nematodes was significantly greater than that of population HP88 and similar to that of population IS–5. At 32 °C, population IS–5 and the hybrid killed *G. mellonella* larvae at a faster rate than population HP88. Both population IS–5 and the hybrid exhibited sensitivity to cold storage at 10 °C. No differences were detected in reproductive potential.

Hybridization as a mean to improve EPN performance was also demonstrated by Shapiro-Ilan, Stuart, and McCoy (2005). They crossed between a highly virulent population (Italian) of *S. carpocapsae* to the pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) and another population (DD–136) which exhibited high levels of heat and desiccation tolerance but poor virulence to that pest. The crosses resulted in enhancement of persistence as well virulence in two out of three populations generated. Heat and desiccation tolerance in all modified populations was more than 2.5-fold greater than the Italian population and not different from the DD–136 population, except one hybrid had lower heat tolerance than DD–136. Mortality of adult *C. caryae* from the modified populations at 2 or 3 days post–treatment was greater than from DD–136 and similar to the Italian population.

Ehlers, Oestergaard, Hollmer, Wingen, and Strauch (2005) attempted selection of *H. bacteriophora* for heat tolerance and cold activity. Analysis of heritability showed a high value ($h^2 = 0.68$) for heat tolerance and a low value ($h^2 = 0.38$) for activity at low temperature. To increase heat tolerance, four rounds of selection were carried out, which increased the mean tolerated temperature from 38.5 to 39.2 °C. The mean temperature at which the IJs of *H. bacteriophora* were active could be

reduced from 7.3 to 6.1 °C after five rounds of selection. However, for unknown reasons, the mean temperature of IJ activity rose during five additional rounds of selection to 7.1 °C. Screening of different isolates of the symbiotic bacterium *Photorhabdus luminescens* for growth at low temperature resulted in several cold–adapted populations from North America, which reached considerable cell density at 6 °C.

To extend the shelf life of a commercial population of *H. bacteriophora*, Strauch et al. (2004) determined the genetic variability of its desiccation tolerance and exploited it for enhancement of this trait by breeding. A hybrid population resulting from crosses of eight *H. bacteriophora* isolates from different geographical origins was used. The desiccation stress was induced by hygroscopic polyethylene glycol (PEG 600) solutions, which lowered the water activity ($a_w$) of this solution, causing removal of water from the IJs. In this study, the influence of an adaptation phase on desiccation tolerance was also investigated. The lowest mean tolerated $a_w$ value (0.85) was achieved with an adaptation phase of 72 h at an $a_w$ value of 0.96. The variance of the desiccation tolerance increased with a reduction in $a_w$ value during adaptation. The heritability of the trait, determined by using homozygous inbred lines, was 0.46 for non–adapted populations (directly exposed to low $a_w$ values), and 0.48 for adapted ones. A negative heterosis effect could be observed for the desiccation tolerance because nearly all of the inbred lines had higher tolerance to desiccation stress than the hybrid strain. Improvement of desiccation tolerance by breeding was only obtained when the adaptation process was included in the selection process, which was related to higher phenotypic variance in the populations after adaptation. A total of eight rounds of selection and breeding were carried out. Without previous adaptation, the mean tolerated $a_w$ value remained almost constant between 0.94 and 0.93. In contrast, when the IJs were adapted prior to exposure to desiccation stress, the tolerated $a_w$ values dropped continuously from 0.89 to 0.81.

In recent years, Mukuka et al. (2010b), Mukuka, Strauch and Ehlers 2010a, Mukuka, Strauch, Al Zainab, and Ehlers (2010), Mukuka, Strauch, Hoppe, and Ehlers (2010), and Mukuka, Strauch, Waeyenberge, Viaene, and Moens (2010) has initiated a GIP for the improvement of heat and desiccation tolerance in *H. bacteriophora*. To enhance heat tolerance, they first characterized the diversity of this trait among 36 populations of *H. bacteriophora* isolated from diverse environments across the globe, as well as 18 hybrid or inbred strains of these bacteria (Mukuka et al., 2010a; Mukuka, Strauch, Waeyenberge et al., 2010). Five populations of *H. indica* and one of *H. megidis* were also included. Nematodes were tested with or without prior adaptation to heat at 35 °C for 3 h. The mean tolerated temperature ranged from 33.3 °C to 40.1 °C for non–adapted populations, and from 34.8 °C to 39.2 °C for adapted ones. *H. indica* was the most tolerant species, followed by *H. bacteriophora* and *H. megidis*. No correlation was found between the assessed tolerance levels with and without adaptation to heat, implying that different genes are involved. Correlation between a population's heat tolerance and the mean annual temperature in its place of origin was weak. A high variability in tolerance among strains and the relatively high heritability ($h^2 = 0.68$) of the heat

tolerance recorded for adapted *H. bacteriophora* provide an excellent foundation for future selective breeding for improved heat tolerance in *H. bacteriophora*.

Desiccation tolerance was evaluated in 43 populations of *Heterorhabditis* spp. and 18 hybrid/inbred populations of *H. bacteriophora* (Mukuka et al., 2010a). Dehydration conditions, measured as $a_w$ values, were produced by treating IJs with different concentrations of the non–ionic polymer PEG 600. Significant interspecific variation was recorded between nematode populations and species. The mean tolerated $a_w$ value (MW50) ranged from 0.90 to 0.95 for non–adapted nematode populations and 0.67 to 0.99 for adapted ones. For selective breeding, only the 10 % most tolerant individuals were used. The lowest $a_w$ value tolerated by this 10 % of the population (MW10) ranged from 0.845 to 0.932 for non–adapted nematode populations and 0.603 to 0.950 for adapted ones. Adaptation significantly increased the desiccation tolerance and a weak correlation was recorded for tolerance with and without adaptation. The nematode populations that were most tolerant to heat or desiccation formed the basis for the foundation of a parental stock produced by cross–breeding and subsequent genetic selection for enhanced tolerance (Mukuka et al., 2010b). In this study, *H. bacteriophora* heat tolerance and desiccation tolerance were significantly increased by cross–breeding tolerant parental strains and successive genetic selection. During the latter process, the selection pressure was constantly increased and only the most tolerant 10 % of the nematode populations were propagated for further selection steps. Assessment of tolerance and selection for both traits were performed with and without prior adaptation to the stress conditions. Eleven rounds of selection were performed to increase heat tolerance. A final overall increase in mean heat tolerance of 5.5 °C was achieved when the nematodes were pre–adapted to the heat stress. For non–adapted tolerance, an increase of 3.0 °C (from 40.1 °C to 43.1 °C) was recorded. For comparison, a commercial population had a mean tolerated temperature after adaptation of 38.2 °C and of 36.5 °C without adaptation. To assess the desiccation tolerance, the mean tolerated $a_w$ value of a population was measured. Cross–breeding the most tolerant populations reduced the $a_w$ value from 0.67 to 0.65 after adaptation, and from 0.9 to 0.7 without prior adaptation. A subsequent six rounds of selection could not increase the tolerance, regardless of whether the nematode had been adapted to the stress.

Monitoring beneficial traits such as infectivity is essential in attempts to genetically improve other traits by crossing tolerant populations or using selective breeding. The fitness of the above–described selected heat– and desiccation–tolerant hybrid strains was evaluated following the selection period (Mukuka, Strauch, Al Zainab et al., 2010; Mukuka, Strauch, Hoppe et al., 2010), in terms of virulence, host penetration and reproductive capacity compared to the commercial population EN 01 of *H. bacteriophora*. Only the heat–tolerant strains were superior or similar in fitness to strain EN 01. The strains with increased desiccation tolerance were generally less fit, possibly reflecting a tradeoff effect of selection for desiccation tolerance. Hybrid strains selected for enhanced tolerance to a stress after adaptation to that stress generally ranked better in terms of fitness than those that were not adapted prior to stress exposure. This could be a result of pleiotropy. The commercial population had the highest reproduction rate per mean number of

nematodes penetrating the host insect, a result of automatic selection of inbred lines with high reproductive potential during the commercial production process in liquid culture.

The effect of stress exposure on the infectivity of heat– and desiccation–tolerant hybrid strains of *H. bacteriophora* was assessed against last instars of *G. mellonella* (Mukuka, Strauch, Al Zainab et al., 2010). Nematode IJs were exposed to desiccation stress at an $a_w$ value of 0.85 for 24 h or to a temperature treatment at 40 °C or 0 °C for 24 h prior to inoculation of five IJs per insect. Hybrid strains resulting from crosses of the three very best heat– or desiccation–tolerant strains and crosses of heat– with desiccation–tolerant strains were compared with a commercial population of *H. bacteriophora*. Exposure to desiccation stress caused a significant reduction in infectivity of all strains, not surpassing 25 % mortality, except one strain that was not affected and achieved 37.5 % mortality. Infectivity of untreated IJs of desiccation–tolerant hybrids differed significantly, with a mean insect mortality of 54 %, ranging from 33.8 to 89.6 %. The mean mortality from infection with heat–tolerant hybrids was significantly higher (78.2 %). Infectivity of the commercial population and two other hybrids were not affected by the heat treatment. Consequently, the authors concluded that the infectivity of heat–tolerant strains is not necessarily affected by low–temperature stress.

*H. bacteriophora* was also subjected to selection for enhancement of nematicidal resistance (Glazer et al., 1997). This is because when applied to the soil, the IJs of this nematode may encounter residual nematicides that will hamper their survival and efficacy. The nematicides used were fenamiphos (an organic phosphate), oxamyl (a carbamate) and avermectin (a biological product). Estimates of heritability ($h^2$ values) for the three nematicides were 0.31, 0.71 and 0.46, respectively. After 11 rounds of selection for resistance to nematicides, resistance increased dramatically. For fenamiphos and avermectin, and eight- to ninefold increase in resistance was recorded, and a 70-fold increase was recorded for oxamyl. When selection was relaxed, resistance to oxamyl and avermectin was stable while a decrease was recorded with fenamiphos. Fitness was retained in all selected populations when evaluated for infectivity. Cross–resistance was displayed for some, but not all of the nematicides tested.

Hashmi et al. (1995) and Hashmi, Hashmi, Glazer, and Gaugler (1998) used genetic engineering as a means of improving heat tolerance of *H. bacteriophora*. They reported the first successful transformation of an EPN. Foreign genes were introduced into *H. bacteriophora* strain HP88 by microinjection using vectors carrying the *C. elegans* genes coding for the roller phenotype and the 16–kDa heat shock protein (*hsp16*). A translational fusion made by inserting *lacZ* in–frame into *hsp16* was expressed in the body musculature, hypodermis, and pharyngeal muscles. Transcription of the *hsp16*/*lacZ* transgenes resulted in the rapid synthesis of detectable levels of β–galactosidase. In another study (Hashmi et al., 1998), successful transformation of the *hsp70* gene was confirmed by Southern blot hybridization and polymerase chain reaction (PCR). Blot studies showed that the transgenic nematodes contain 5 to 10 copies per genome of the introduced *hsp70*. Transcripts of *hsp70* mRNA were detected in both wild–type and transgenic

nematodes. Transcripts increased several fold in transgenic nematodes upon heat shock. IJs of both transgenic and wild–type nematodes were exposed to a sublethal heat treatment (35 °C) for 2 h followed by a normally lethal heat treatment (40 °C) for 1 h. More than 90 % of the transgenic nematodes survived the heat treatment, compared to 2–3 % of the wild–type strain. Overexpression of *hsp70* resulted an enhanced thermotolerance in the transgenic nematodes, which displayed normal growth and development. Furthermore, the transgenic strain was released in turf grass field microplots in the spring, summer, and fall of 1996 (Gaugler, Wilson, & Shearer, 1997), in accordance with the regulatory procedures at the federal, state, university and local levels needed for field release in the USA. As predicted, persistence of the transgenic and wild–type populations did not differ. This risk–assessment study supports the view that the transgenic nematode population is an unlikely environmental threat.

Vellai et al. (1999) reported successful transformation of the yeast desiccation–related gene encoding trehalose–6–phosphate synthase into the nematode *S. feltiae*. The transformed lines were able to survive well in increased concentrations of NaCl in M9 storage solution, while rapid mortality was recorded in the wild–type population. This study demonstrated the ability to transform steinernematids. However, the bioassays verifying the increase in osmotic–pressure tolerance were performed with a population of adult nematodes, not with IJs. The authors also suggested using the LEA–encoding genes (Browne, Tunnacliffe, & Burnell, 2002; Gal et al., 2004) as candidates for transformation, or the gene promoter, to enhance desiccation tolerance (Vellai et al.).

Unlike *Heterorhabditis*, species of *Steinernema* showed less genetic improvement. Selection for cold tolerance of *S. feltiae* together with its bacterial symbiont, *Xenorhabdus bovienii*, had been conducted by Grewal, Gaugler, and Wang (1996) by repeated passage through *G. mellonella* larvae at 15 °C. Nematode virulence (total insect mortality and speed of kill) and establishment (initiation of nematode development following penetration) were evaluated after 6 (=12–24 generations) and 12 (=24–36 generations) passages. Cold selection enhanced nematode virulence at the cooler temperatures. Virulence measured as total insect mortality at 8 °C improved by 5.3- and 6.6-fold after 6 and 12 passages, respectively. Only small improvements (1.2- to 1.5-fold) were observed in speed of kill. Nematode establishment improved at all temperatures after 12 passages; the highest increase, nine-fold, was observed at 8 °C.

Bal et al. (2014) genetically selected the “ambush” foraging *S. carpocapsae* for enhanced dispersal in the absence of hosts by capturing the fastest and farthest reaching IJs emanating from a nematode–infected *G. mellonella* cadaver, in soil. The selected *S. carpocapsae* showed positive response to selection for dispersal with 13–23 and 21–37 fold increase in the percent IJs dispersing to the farthest distance from the source cadaver, after five and ten rounds of selection, respectively. There was also a significant increase in the average displacement of the selected lines (6.85–7.54 cm/day) than the foundation population (5.54 cm/day) maintained by passing through *G. mellonella* larvae in Petri dishes. The overall mean realized heritability for dispersal was 0.60. The farthest reaching IJs of the

selected lines comprised more males (72 %) than the foundation population (44 %) at most time points. Trade–offs associated with enhanced dispersal included reduced reproduction capacity and nictation ability, a trait associated with ambush foraging.

Salame et al. (2010) bred a heterogeneous population of the EPN *S. feltiae* for tolerance to both rapid and slow desiccation. The nematodes were selected for tolerance of rapid desiccation by exposing IJs to ambient conditions [22–25 °C; 50–65 % relative humidity (RH)] for 100 min. A survival rate of 80–90 % was reached after 10 selection cycles. To select for tolerance of slow desiccation, the IJs were exposed to 97 % RH for 72 h, followed by exposure to 85 % RH for an additional 72 h. A high survival rate (>85 %) was obtained after 20 selection cycles. No reduction in fitness was detected in the selected populations. Nevertheless, the population selected for slow desiccation was more tolerant of heat stress than the foundation population.

## 2.4 Trait Stability

Genetic stability of genetically selected lines has been questioned and evaluated from the earliest attempts to improve nematode performance by genetic means. Gaugler and Campbell (1989), who selected *S. feltiae* for host finding, indicated that "relaxation of selection pressure produced a gradual decrease in host–finding". Similarly, in other studies where trait stability was evaluated after relaxation of the selection regime, a certain reduction was reported (see Table 2.1). Nevertheless, genetic deterioration has also been reported in non–selected EPN populations subjected to continuous laboratory or industrial propagation (Gaugler & Campbell, 1991; Stuart & Gaugler, 1996). Shapiro-Ilan, Glazer, and Segal (1996) reported that the heat–tolerance trait in newly isolated *H. bacteriophora* population IS–5 remained stable after 12 passages of the culture in *G. mellonella*. Other fitness measures (infectivity, reproduction and storage at 25 °C) retained their initial levels. In contrast, Wang and Grewal (2002) reported rapid deterioration of environmental tolerance (to heat, desiccation, and UV) and reproductive potential for *H. bacteriophora* population GPS11 during maintenance in the laboratory.

Similar trait changes were observed by Bilgrami, Gaugler, Shapiro-Ilan, and Adams (2006), who studied the stability of traits important for biological control in *H. bacteriophora* and *S. carpocapsae*; they reported that 20 serial passages in *G. mellonella* results in impaired performance of both nematode species. Virulence, heat tolerance and fecundity deteriorated in all experimental *H. bacteriophora* lines, and four out of five experimental lines deteriorated in host–finding ability. All *S. carpocapsae* experimental lines deteriorated in heat tolerance and nictation, and four out of five experimental lines showed decreased reproductive capacity, whereas virulence declined in two experimental lines. They tested whether trait deterioration was due to changes in the nematode, bacterium, or both symbiotic partners by exchanging nematodes or bacteria from control populations with nematodes or bacteria from the most deteriorated experimental lines and assessing trait recovery. The source of deterioration varied according to the trait, but only the bacterial

partner played a role in trait reduction for every trait and species, whereas the nematode was the main source only for *S. carpocapsae* nictation.

Hiltpold, Baroni, Toepfer, Kuhlmann, and Turlings (2010a) who enhance *H. bacteriophora* responsiveness towards (*E*)–β–caryophyllene (EβC) (see above) reported that this process resulted in a slight but significant reduction in infectiousness of the the selected population to the target insect the WCR. Yet, this apparent cost was largely compensated for by the higher responsiveness to the root signal. In further study, Hiltpold, Baroni, Toepfer, Kuhlmann, and Turlings (2010b) showed that the selection process had no negative effect on establishment and persistence of field–released EPN.

Bai, Shapiro-Ilan, Gaugler, and Hopper (2005) suggested stabilizing beneficial traits in *H. bacteriophora* through the creation of genetically homozygous inbred lines that can deter beneficial trait decline. Trait stability was evaluated following serial culturing of three inbred lines and the foundation population in *G. mellonella*. Laboratory data indicated that serial culture of the foundation population (16 passages) results in an over 30 % loss in traits deemed beneficial for biological pest suppression, i.e., virulence to an insect host *Diaprepes abbreviates* L. (Coleoptera: Curculionidae), reproductive capacity, heat tolerance (at 38 °C), and host–seeking ability. In contrast, the inbred lines were impervious to declines in all beneficial traits. A greenhouse test targeting *D. abbreviatus* provided additional evidence that the biocontrol efficacy of the inbred lines remains stable during serial culture.

To stabilize the progress made by selective breeding for desiccation tolerance of *H. bacteriophora*, Anbesse, Sumaya, Dörfler, Strauch, and Ehlers (2013a) tested selection during liquid culturing vs. propagation in host insects. After release of the selection pressure, the tolerance was monitored over additional reproductive cycles *in vivo* and *in vitro* to compare the stability of the trait. Furthermore, they tested whether the virulence of the selected populations was impaired. Exposure to desiccation stress prior to propagation, *in vivo* or *in vitro*, resulted in increased desiccation tolerance. When selection pressure was released, the gained tolerance was lost again during *in vivo* production, whereas the tolerance was maintained at a high level in liquid–cultured EPNs. Anbesse, Sumaya, Dörfler, Strauch, and Ehlers (2013b) further evaluated the stability of *H. bacteriophora* populations selected for heat tolerance using an inbred line reared in liquid culture. After release of the selection pressure, the tolerance was monitored for 15 additional reproductive cycles to determine the stability of the trait. Virulence of the selected populations was assessed to check for negative tradeoff effects. Heat tolerance was successfully increased in *H. bacteriophora* propagated *in vivo* (from 39.03 to 40.85 °C) and *in vitro* (from 39 to 40 °C), but could only be maintained in populations which were serially reared in liquid culture. Anbesse, Strauch, and Ehlers (2012) also investigated possible heterosis effects in desiccation and heat tolerance after cross–breeding of homozygous inbred lines of *H. bacteriophora*. Higher desiccation tolerance of the heterozygous progeny compared to the homozygous inbred lines was recorded, indicating that heterosis is a possible means for further improvement of this trait. In contrast, the heat tolerance of the heterozygous offspring was lower than that of the homozygous population.

When *H. bacteriophora* is cultured *in vivo*, reproduction by cross–fertilization is possible. In *in–vitro* culture, males and females cannot mate and reproduction occurs solely by self–fertilizing hermaphrodites resulting in homozygous inbred lines. Therefore, the studies described above suggest that liquid culture could highly improve and stabilize beneficial traits of heterorhabditid EPNs through selective breeding. Selection using liquid culture technology is thus superior to *in–vivo* propagation in sustaining beneficial traits in *H. bacteriophora*, not only for selective breeding but also for mass production.

Adhikari et al. (2009) generated transcriptional profiles of two experimental lines of *H. bacteriophora*, identified the differentially expressed genes (DEGs) and validated their differential expression in the deteriorated line. The expression–profiling study was performed between experimental lines L5M and OHB of *H. bacteriophora* with probes for 15,220 ESTs from the *H. bacteriophora* transcriptome. Microarray analysis showed 1,185 DEGs comprised of of 469 down– and 716 upregulated genes in nematodes with deteriorated traits. Analysis of the DEGs showed that trait deterioration involves massive changes in the transcripts encoding enzymes involved in metabolism, signal transduction, virulence and longevity. We observed a pattern of reduced expression of enzymes related to primary metabolic processes and induced secondary metabolism. Expression of 16 DEGs in deteriorated–trait nematodes was validated by quantitative reverse transcription–PCR, which revealed similar expression kinetics for all of the genes tested, as shown by microarray.

One of the most powerful tools for genetic analysis and improvement is the induction of mutations (Fodor et al., 1990). The first induction and characterization of mutants of *H. bacteriophora* was reported by Zioni, Glazer, and Segal (1992). A homozygous inbred line was used as the base population for mutagenesis and genetic analysis. Mutagenesis was induced by exposing young hermaphrodites to 0.05 M ethyl–methanesulfonate, and a dumpy (dpy) mutant (*Hdpy–1*) was isolated. Morphological analysis revealed distortion of the head region in adults as well as in IJs. Backcrosses with the wild–type population and genetic analysis revealed that the mutation is recessive. Later on, more recessive dpy mutants—*Hdpy–2 and Hdpy–3*—were isolated and characterized (Koltai et al., 1994). Complementation tests indicated that each of the three mutations affects different genes. The *Hdpy–2* mutant was used as a genetic marker to validate crosses between heat–tolerant and heat–sensitive populations (Shapiro-Ilan et al., 1997) as described above.

Tomalak (1994c) described the first morphological and behavioral mutant in *S. feltiae*. The mutation was spontaneous and occurred in a single gene locus designated *Sfdpy–1*. Action of the new allele was only clearly expressed in IJs. The resulting morphology was classified as ‘dumpy’ due to the significantly reduced ratio of the nematode’s body length to maximum diameter. The identified gene was sex–linked and the new mutant allele remained recessive to the wild–type counterpart responsible for normal morphology. Aside from altering the morphology, pleiotropic action of the dpy allele affected nematode movement and infectivity to insect hosts. The mean dispersion distance of mutant juveniles and their infectivity to *G. mellonella* and *L. solani* larvae were significantly reduced compared to those

of wild–type nematodes. Revertant individuals that were occasionally isolated from dpy strains regained the ability to move quickly and dispersed even further than the juveniles from the parental strains. They were also more effective at penetrating the hemocoel of *L. solani* larvae. However, the numbers of infected insects did not significantly differ from those observed for wild–type ScP and SN populations. Following this study, Tomalak (1997) described morphological (Tomalak & Mráček, 1998) and genetic analyses of eight additional mutants of *S. feltiae*. These mutations were also found to be recessive and sex–linked.

In recent years, a new tool for the induction of mutations and genetic modifications has been developed, particularly for *C. elegans* research. Ciche and Sternberg (2007) developed the use of RNAi in heterorhabditid nematodes. This approach was further used by Moshayov, Koltai, and Glazer (2013) to silences genes that are presumably related to the recovery process from the infective to parasitic stage. Understanding of the recovery process and its genetic basis may lead to improvement in nematode recovery. This is an important step in nematode production and can increase its efficiency

Recently, a method to edit the *C. elegans* genome using the clustered, regularly interspersed, short palindromic repeats (CRISPR) RNA–guided Cas9 nuclease has been developed (Dickinson, Ward, Reiner, & Goldstein, 2013; Friedland et al., 2013). Cas9 was able to induce DNA double–strand breaks with specificity for targeted sites, and these breaks could be efficiently repaired by homologous recombination. By supplying engineered homologous repair templates, the researchers generated *gfp* knock–ins and targeted mutations. The results outline a flexible methodology to produce essentially any desired modification in the nematode genome quickly and at low cost. This technology is an important addition to the array of available genetic techniques and can be utilized in EPNs.

Genomic and bioinformatic tools are now available for whole–genome analysis. Bai et al. (2007, 2013) compared *H. bacteriophora* GPS11 expressed sequence tags (ESTs) to the ESTs of animal–parasitic, human–parasitic, plant–parasitic, and free–living nematodes: 127 of them were identified as previously undescribed ESTs, of which 119 had homologs in ESTs and 8 had homologs in proteins of free–living nematodes. These ESTs were assigned putative functions in transcription, signal transduction, cell–cycle control, metabolism, information processing, and cellular processes, thereby providing better insight into *H. bacteriophora* metabolism, sex determination, and signal transduction. In addition, 36 *H. bacteriophora* ESTs had significant similarities to ESTs of parasitic nematodes, but not to ESTs or proteins of free–living nematodes species. Among these were ESTs encoding a centrin, an ankyrin–repeat–containing protein, and a nuclear hormone receptor. The analysis also revealed that parasitic nematode–specific ESTs in this *H. bacteriophora* dataset have more homologs in animal–parasitic nematodes than in those parasitizing humans or plants.

Tyson et al. (2012) recently conducted a molecular analysis of desiccation–tolerance mechanisms in the anhydrobiotic nematode *Panagrolaimus superbus* (Rhabditida: Panagrolaimidae) using ESTs, and revealed a series of candidate genes that may have an important role in stress–tolerance mechanisms. Since

this nematode is closely related to EPN (same order) this information will be useful for understanding the basis of stress tolerance of these organisms. In addition, transcriptomic analysis of *H. bacteriophora* (Adhikari et al., 2009) and *S. carpocapsae* (Hao, Montiela, Abubuckerb, Mitrevab, & Simoes, 2010) also listed genes with relevance to stress tolerance. In a recent study, (Yaari et al., 2015) analyzed the transcriptomes of various steinernematid species with various tolerance capabilities to desiccation and heat stresses. This accumulated information will provide the basis for improvement of EPNs by genetic engineering.

Unlike many other beneficial organisms which are subjected to genetic improvement like agricultural plants and animals, EPNs are lacking of useful tools. That includes tools for characterization of heterogenicity/homogenicity of a population, markers to follow transfer or enhancement/degradation of traits as well identified beneficial genes which can be transferred between populations by crosses or molecular means.

## 2.5 Future Prospects of Genetic Improvement in Entomopathogenic Nematodes

Genetic approaches still hold great promise for improvement of EPNs. Studies have clearly shown that the use of classical and advanced genetic techniques can significantly enhance EPN performance (Table 2.1). However, if we look at the scheme established by Hoy (1990), it is evident that most of the research efforts to date fall within the framework of the second step, i.e., "determination of variability and genetic improvement by selection, mutagenesis or gene transfer". Unlike many other beneficial organisms which are subjected to genetic improvement like agricultural plants and animals, EPN are lacking of useful tools. That includes tools for characterization of heterogenicity/homogenicity of a population (RFLP, AFLP, SNPs, SSRs– see general review by Freeland, 2005), markers to follow transfer or enhancement/degradation (Molecular, morphological and QTLs– Freeland) of traits as well identified beneficial genes which can be transferred between populations by crosses or molecular means. Fundamental research into the genetic architecture of key traits, such as infectivity, stress tolerance and reproduction, is needed. The new genomic, proteomic and bioinformatic technologies should be adapted for EPN genetic research (Dillman, Mortazavi, & Sternberg, 2012). Considering that the genome of *H. bacteriophora* (TTO1 population) has been sequenced (Bai et al., 2013), new tools for genome editing (RNAi, CRISPR–Cas9) may be used for research as well as for the development of genetically improved nematodes. The present review shows that the development of genetically improved "products" stops, for the most part, in the laboratory, with only a very few (Gaugler et al., et al., 1997; Grewal et al., 1993) being tested in field trials. The perception is that improvement of a particular trait (infectivity, stress tolerance, etc.) has yet to be proven under natural conditions.

## References


Adhikari, B. N., Lin, C., Bai, X., Ciche, T. A., Grewal, P. S., Dillman, A. R., et al. (2009). Transcriptional profiling of trait deterioration in the insect pathogenic nematode *Heterorhabditis bacteriophora. BioMed Central (BMC) Genomics, 10*, 609–616.

Allen, H. W. (1954). Propagation of *Horogenes molestae*, a parasite of the oriental fruit moth, on the potato tuber–worm. *Journal of Economic Entomology, 47*, 278–281.

Anbesse, S., Strauch, O., & Ehlers, R.-U. (2012). Genetic improvement of the biological control nematode *Heterorhabditis bacteriophora* (Rhabditidomorpha: Heterorhabditidae): Heterosis effect enhances desiccation but not heat tolerance. *Biocontrol Science and Technology, 22*, 1035–1045.

Anbesse, S., Sumaya, H. N., Dörfler, V. A., Strauch, O., & Ehlers, R.-U. (2013a). Selective breeding for desiccation tolerance in liquid culture provides genetically stable inbred lines of the entomopathogenic nematode *Heterorhabditis bacteriophora. Applied Microbiology and Biotechnology, 97*, 731–739.

Anbesse, S., Sumaya, H. N., Dörfler, V. A., Strauch, O., & Ehlers, R.-U. (2013b). Stabilization of heat tolerance traits in *Heterorhabditis bacteriophora* through selective breeding and creation of inbred lines in liquid culture. *BioControl, 58*, 85–93.

Bai, C., Shapiro-Ilan, D. I., Gaugler, R., & Hopper, K. R. (2005). Stabilization of beneficial traits in *Heterorhabditis bacteriophora* through creation of inbred lines. *Biological Control, 32*, 220–227.

Bai, X., Grewal, P. S., Hogenhout, S. A., Adams, B. J., Ciche, T. A., Gaugler, R., et al. (2007). Comparative analysis of *Heterorhabditis bacteriophora* expressed sequence tags (ESTs). *Journal of Parasitology, 93*, 1343–1349.

Bai, X., Adams, B. J., Ciche, T., Clifton, A., Gaugler, R., Kim, K. S., et al. (2013). A lover and a fighter: The genome sequence of an entomopathogenic nematode *Heterorhabditis bacteriophora. PLoS ONE, 8*, e69618.

Bal, K. H., Michel, P. A., & Grewal, S. P. (2014). Genetic selection of the ambush foraging entomopathogenic nematode, *Steinernema carpocapsae* for enhanced dispersal and its associated trade–offs. *Evolutionary Ecology, 28*, 923–939.

Barrett, J. (1991). Anhydrobiotic nematodes. *Agricultural Zoology Reviews, 4*, 161–176.

Baur, M. E., Kaya, H. K., & Thurston, G. S. (1995). Factors affecting entomopathogenic nematode infection of *Plutella xylostella* on a leaf surface. *Entomologia Experimentalis et Applicata, 77*, 239–250.

Bilgrami, A. L., Gaugler, R., Shapiro-Ilan, D. I., & Adams, B. J. (2006). Source of trait deterioration in entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during *in vivo* culture. *Nematology, 23*, 397–409.

Box, H. E. (1956). Battle against Venezuela's cane borer. I. Preliminary investigations and the launching of a general campaign. *Sugar (New York), 51*, 25–27.

Browne, J., Tunnacliffe, A., & Burnell, A. (2002). Plant desiccation gene found in nematode. *Nature, 416*, 3.

Burnell, A. M., & Dowds, B. C. A. (1996). The genetic improvement of entomopathogenic nematodes and their symbiotic bacteria: Phenotypic targets, genetic limitations and an assessment of possible hazards. *Biocontrol Science and Technology, 6*, 435–447.

Burnell, A. M., & Tunnacliffe, A. (2011). Gene induction and desiccation stress in nematodes. In R. N. Perry & D. A. Wharton (Eds.), *Molecular and physiological basis of nematode survival* (pp. 126–156). Wallingford, U.K.: CAB International.

Caroli, L., Glazer, I., & Gaugler, R. (1996). Entomopathogenic nematode infectivity assay: Multi variable comparison of penetration into different hosts. *Biocontrol Science and Technology, 6*, 227–233.

Chen, S., Gallop, N., & Glazer, I. (2005). Cross stress tolerance and expression of stress–related proteins in osmotically desiccated entomopathogenic nematode *Steinernema feltiae* IS–6. *Parasitology, 131*, 695–703.

Chen, S., Glazer, I., Gollop, N., Cash, P., Argo, E., Innes, A., et al. (2005). Proteomic analysis of the entomopathogenic nematode *Steinernema feltiae* IS–6 IJs under evaporative and osmotic stresses. *Molecular Biochemical Parasitology, 145*, 195–204.

Ciche, T. A., & Sternberg, P. W. (2007). Postembryonic RNAi in *Heterorhabditis bacteriophora*: a nematode insect parasite and host for insect pathogenic symbionts. *BioMed Central (BMC) Developmental Biology, 7*, 101–111.

Close, T. J. (1996). Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. *Physiological Plantarum, 97*, 795–803.

Crowe, J. H., & Madin, K. A. C. (1975). Anhydrobiosis in nematodes: Evaporative water loss and survival. *Journal of Experimental Zoology, 193*, 323–334.

Dickinson, D. J., Ward, J. D., Reiner, J. D., & Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* genome using *Cas9*–triggered homologous recombination. *Nature Methods*.

Dillman, A. R., Mortazavi, A., & Sternberg, W. P. (2012). Incorporating genomics into the toolkit of nematology. *Journal of Nematology, 44*, 191–205.

Dure, L. (1993). Plant response to cellular dehydration during environmental stress: structural motifs in LEA proteins. In T. J. Close & E. A. Bray (Eds.), *Current topics in plant physiology* (Vol. 10, pp. 91–103). Rockville, MD: American Society of Plant Physiologists.

Ehlers, R.-U., Oestergaard, J., Hollmer, S., Wingen, M., & Strauch, O. (2005). Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode–bacterium complex *Heterorhabditis bacteriophora–Photorhabdus luminescens. BioControl, 50*, 699–716.

Fodor, A., Vecseri, G., & Farkas, T. (1990). *Caenorhabditis elegans* as a model for the study of entomopathogenic nematodes. In R. Gaugler & H. K. Kaya (Eds.), *Entomopathogenic nematodes in biological control* (pp. 249–269). Wallingford, U.K.: CAB International.

Freeland, R. J. (2005). *Molecular ecology*. West Sussex, England: Wiley.

Friedland, A. E., Tzur, B. Y., Esvelt, M. K., Colaiácovo, P. M., Church, M. G., & Calarco, A. J. (2013). Heritable genome editing in *C. elegans* via a CRISPR–*Cas9* system. *Nature Methods*.

Gal, T., Glazer, I., & Koltai, H. (2003). Differential gene expression during desiccation stress in *Steinernema feltiae* IS6. *Journal of Parasitology, 89*, 761–766.

Gal, T., Glazer, I., & Koltai, H. (2004). An LEA3 family member is involved in survival of *C. elegans* during exposure to dehydration stress. *FEBS Letters, 577*, 21–26.

Gal, T., Glazer, I., & Koltai, H. (2005). Stressed worms: Responding to the post genomic era. *Molecular and Biochemical Parasitology, 143*, 1–5.

Gal T., Glazer I., Sherman A., & Koltai H. (2005). Protein interaction of Nucleosome Assembly Protein–1 and Casein Kinase 2 during desiccation response in the insect–killing nematode *Steinernema feltiae* IS–6. *Journal of Parasitology* 91, 691–693.

Gal, T., Solomon, A., Glazer, I., & Koltai, H. (2001). Alteration in the levels of glycogen and glycogen synthase transcripts during desiccation in the insect–killing, highly desiccation tolerant nematode *Steinernema feltiae* IS–6. *Journal of Parasitology, 87*, 725–732.

Gaugler, R. (1987). Entomogenous nematodes and their prospects for genetic improvement. In K. Maramorosch (Ed.), *Biotechnology in invertebrate pathology and cell culture* (pp. 457–484). San Diego, CA: Academic.

Gaugler, R., & Campbell, J. F. (1989). Selection for host–finding in *Steinernema feltiae. Journal of Invertebrate Pathology, 54*, 363–372.

Gaugler, R., & Campbell, J. F. (1991). Selection for enhanced host–finding of scarab larvae (Coleoptera: Scarabaeidae) in an entomopathogenic nematode. *Environmental Entomology, 20*, 700–706.

Gaugler, R., Campbell, J. F., & McGuire, T. R. (1990). Fitness of a genetically improved entomopathogenic nematode. *Journal of Invertebrate Pathology, 56*, 106–l16.

Gaugler, R., Wilson, M., & Shearer, P. (1997). Field release and environmental fate of a transgenic entomopathogenic nematode. *Biological Control, 9*, 75–80.

Glazer, I. (1992). Survival and efficacy of *Steinernema carpocapsae* in an exposed environment. *Biocontrol Science and Technology, 2*, 101–107.

Glazer, I. (2002). Survival biology. In R. Gaugler (Ed.), *Entomopathogenic nematodes* (pp. 169–187). Wallingford, U.K.: CABI Publishing.

Glazer, I., Gaugler, R., & Segal, D. (1991). Genetics of the entomopathogenic nematode *Heterorhabditis bacteriophora* strain HP88: The diversity of beneficial traits. *Journal of Nematology, 23*, 324–333.

Glazer, I., Kozodoi, E., Hashmi, G., & Gaugler, R. (1996). The entomopathogenic nematode *Heterorhabditis bacteriophora* IS–5; a heat tolerant isolate from Israel. *Nematologica, 42*, 481–492.

Glazer, I., Salame, L., & Segal, D. (1997). Genetic enhancement of nematicidal resistance of entomopathogenic nematodes. *Biocontrol Science and Technology, 7*, 499–512.

Grewal, P. S., Ehlers, R.-U., & Shapiro-Ilan, D. I. (2005). *Nematodes a biocontrol agents*. Oxfordshire, U.K.: CABI Publishing.

Grewal, P. S., Tomalak, M., Keil, C. B. O., & Gaugler, R. (1993). Evaluation of a genetically selected strain of *Steinernema feltiae* against the mushroom sciarid *Lycoriella mali. Annals of Applied Biology, 123*, 695–702.

Grewal, P. S., Gaugler, R., & Wang, Y. (1996). Enhanced cold tolerance of the entomopathogenic nematode *Steinernema feltiae* through genetic selection. *Annals of Applied Biology, 129*, 335–341.

Hao, Y.-J., Montiela, R., Abubuckerb, S., Mitrevab, M., & Simoes, N. (2010). Transcripts analysis of the entomopathogenic nematode *Steinernema carpocapsae* induced in vitro with insect haemolymph. *Molecular & Biochemical Parasitology, 169*, 79–86.

Hashmi, S., Hashmi, G., & Gaugler, R. (1995). Genetic transformation of an entomopathogenic nematode by microinjection. *Journal of Invertebrate Pathology, 66*, 293–296.

Hashmi, S., Hashmi, G., Glazer, I., & Gaugler, R. (1998). Thermal response of *Heterorhabditis bacteriophora* transformed with the *Caenorhabditis elegans hsp70* encoding gene. *Journal of Experimental Zoology, 281*, 164–170.

Havron, A., Kenan, G., & Rosen, D. (1991). Selection for pesticide resistance in *Aphytis*. II. A. lingnanensis, a parasite of the California red scale. *Entomologia Experimentalis et Applicata, 61*, 221–228.

Havron, A., Rosen, D., Prag, H., & Rossler, Y. (1991). Selection for pesticide resistance in *Aphytis. I. holoxanthas*, a parasite of the Florida red scale. *Entomologia Experimentalis et Applicata, 61*, 229–235.

Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U., & Turlings, T. C. J. (2010a). Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. *Journal of Experimental Biology, 213*, 2417–2423.

Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U., & Turlings, T. C. J. (2010b). Selective breeding of entomopathogenic nematodes for enhanced attraction to a root signal did not reduce their establishment or persistence after field release. *Plant Signaling and Behavior, 5*, 1450–1452.

Hoy, M. A. (1984). Genetic improvement of a biological control agent: Multiple pesticide resistance and nondiapause in *Metaseiulus occidentalis* (Nesbitt) (Pytoseiidae). In: D. A., Griffiths & C. E. Bowman (Eds.), *Proceedings VI International Congress of Acarology*, *Acarology VI*, Vol. 2. (pp. 673–679). New York, NY: Ellis Horwood Ltd., Halsted Press.

Hoy, M. A. (1985a). Improving establishment of arthropod naturel enemies. In: M. A. Hoy & D. C. Herzog (Eds.). *Biological control in agricultural IPM systems* (pp. 151–166). Orlando, FL: Academic Press.

Hoy, M. A. (1985b). Integrated mite management for California almond orchards. In: W. Helle & M. W. Sabelis (Eds.), *Spider mites, their biology, natural enemies and control*, Vol. IB (pp. 299–310). Amsterdam, The Netherlands: Elsevier Science Publ.

Hoy, M. A. (1985c). Recent advances in genetics and genetic improvement of the Phytoseiidae. *Annual Review of Entomology, 30*, 345–370.

Hoy, M. A. (1986). Use of genetic improvement in biological control. *Agriculture, Ecosystems and Environment, 15*, 109–119.

Hoy, M. A. (1990). Genetic improvement of arthropod natural enemies: Becoming a conventional tactic? *UCLA Symposia on Molecular and Cellular Biology, 112*, 405–417.

Hoy, M. A., & Cave, F. E. (1991). Genetic improvement of a parasitoid: Response of Trioxys-pallidus to laboratory selection with Azinphosmethyl. *Biocontrol Science and Technology, 1*, 31–41.

Hoy, M. A., Conley, J., & Robinson, W. (1988). Cyhexatin and fenbutatin–oxide resistance in Pacific spider mite (Atari: Tetranychidae): Stability and mode of inheritance. *Journal of Economic Entomology, 81*, 57–64.

Hoyt, S. C. (1969). Integrated chemical control of insects and biological control of spider mites on apple in Washington. *Journal of Economic Entomology, 62*, 74–86.

Ingram, J., & Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 147*, 377–403.

Ishibashi, N., Tojo, S., & Hatate, H. (1987). Desiccation survival of *Steinernema feltiae* str. DD–136 and possible desiccation protectants for foliage application of nematodes. In: N. Ishibashi (Ed.). *Recent advances in biological control of insect pests by entomogenous nematodes in Japan* (pp. 139–144). Japan: Ministry of Education.

Johnigk, S. A., Hollmer, S., Strauch, O., Wyss, U., & Ehlers, R.-U. (2002). Heritability of the liquid culture mass production potential of the entomopathogenic nematode *Heterorhabditis bacteriophora. Biocontrol Science and Technology, 12*, 267–276.

Kaya, H. K. (1990). Soil ecology. In R. Gaugler & H. K. Kaya (Eds.), *Entomopathogenic nematodes in biological control* (pp. 93–115). Boca Raton, FL: CRC Press.

Koltai, H., Glazer, I., & Segal, D. (1994). Phenotypic and genetic analysis of two mutants of *Heterorhabditis bacteriophora* strain HP88. *Journal of Nematology, 26*, 32–39.

Kung, S. P., & Gaugler, R. (1990). Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology, 55*, 401–406.

Kung, S. P., Gaugler, R., & Kaya, H. K. (1990). Influence of soil pH and oxygen on entomopathogenic nematode persistence. *Journal of Nematology, 22*, 440–445.

Liu, Q.-Z., & Glazer, I. (2000). Factors affecting desiccation survival of the entomopathogenic nematodes, *Heterorhabditis bacteriophora* HP88. *Phytoparasitica, 28*, 331–340.

Menti, H., Wright, D. J., & Perry, R. N. (1997). Desiccation survival of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *Journal of Helminthology, 71*, 41–46.

Moshayov, A., Koltai, H., & Glazer, I. (2013). Molecular characterization of the recovery process in the entomopathogenic nematode *Heterorhabditis bacteriophora. International Journal of Parasitology, 43*, 843–852.

Mukuka, J., Strauch, O., & Ehlers, R.-U. (2010a). Variability in desiccation tolerance among different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora. Nematology, 12*, 711–720.

Mukuka, J., Strauch, O., & Ehlers, R.-U. (2010b). Improvement of heat and desiccation tolerance in *Heterorhabditis bacteriophora* through cross–breeding of tolerant strains and successive genetic selection. *BioControl, 55*, 511–521.

Mukuka, J., Strauch, O., Al Zainab, H. M., & Ehlers, R.–. U. (2010). Effect of temperature and desiccation stress on infectivity of stress tolerant hybrid strains of *Heterorhabditis bacteriophora. Russian Journal of Nematology, 18*, 111–116.

Mukuka, J., Strauch, O., Hoppe, C., & Ehlers, R.-U. (2010). Fitness of heat and desiccation tolerant hybrid strains of *Heterorhabditis bacteriophora* (Rhabditidomorpha: Heterorhabditidae). *Journal of Pest Sciences, 83*, 281–287.

Mukuka, J., Strauch, O., Waeyenberge, L., Viaene, N., Moens, M., & Ehlers, R.-U. (2010). Heat tolerance among different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora. BioControl, 55*, 423–434.

Norton, D. C. (1978). *Ecology of plant–parasitic nematodes*. Westchester, NY: John Wiley & Sons.

O'Leary, S. A., Stack, C. M., Chubb, M. A., & Burnell, A. M. (1998). The effect of day of emergence from the insect cadaver on the behavior and environmental tolerances of infective juveniles of the entomopathogenic nematode *Heterorhabditis megidis* (strain UK211). *Journal of Parasitology, 84*, 665–672.

Pielou, D. P., & Glasser, R. F. (1952). Selection for DDT resistance in a beneficial insect parasite. *Science, 115*, 117–118.

Ram, A., & Sharma, A. K. (1977). Selective breeding for improving the fecundity and sex ratio of *Thrichogramma fasciatum* (Perkins) (Trichogrammatidae: Hymenoptera), an egg parasite of lepidopterous hosts. *Entomology, 2*, 133–137.

Ricci, M., Glazer, I., & Gaugler, R. (1996). Entomopathogenic nematode infectivity assay: Comparison of laboratory bioassays. *Biocontrol Science and Technology, 6*, 235–245.

Roush, R. T., & Hoy, M. A. (1981). Genetic improvement of *Metaseiulus occidentalis*: Selection with methomyl, dimethoate, and carbaryl and genetic analysis of carbaryl resistance. *Journal of Economic Entomology, 74*, 138–141.

Salame, L., Glazer, I., Chubinishvilli, M. T., & Chkhubianishvili, T. (2010). Genetic improvement of the desiccation tolerance and host–seeking ability of the entomopathogenic nematode *Steinernema feltiae. Phytoparasitica, 38*, 359–368.

Segal, D., & Glazer, I. (1998). Genetic approaches for enhancing beneficial traits in entomopathogenic nematodes. *Japanese Journal of Nematology, 28*, 101–107.

Segal, D., & Glazer, I. (2000). Genetics for improving biological control agents: The case of entomopathogenic nematodes. *Crop Protection, 19*, 685–689.

Shapiro-Ilan, D. I., Glazer, I., & Segal, D. (1996). Trait stability and fitness of the heat tolerant entomopathogenic nematode *Heterorhabditis bacteriophora* IS5 strain. *Biological Control, 6*, 238–244.

Shapiro-Ilan, D. I., Glazer, I., & Segal, D. (1997). Genetic improvement of heat tolerance in *Heterorhabditis bacteriophora* through hybridization. *Biological Control, 8*, 153–159.

Shapiro-Ilan, D. I., Stuart, J. R., & McCoy, W. C. (2005). Targeted improvement of *Steinernema carpocapsae* for control of the pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) through hybridization and bacterial transfer. *Biological Control, 34*, 215–221.

Shapiro-Ilan, D. I., Han, R., & Dolinksi, C. (2012). Entomopathogenic nematode production and application technology. *Journal of Nematology, 44*, 206–217.

Simmonds, F. J. (1947). Improvement of the sex ratio of a parasite by selection. *Canadian Entomology, 79*, 41–44.

Simons, W. R., & Poinar, G. O., Jr. (1973). The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. *Journal of Invertebrate Pathology, 22*, 228–230.

Solomon, A., Paperna, I., & Glazer, I. (1999). Desiccation survival of the entomopathogenic nematode *Steinernema feltiae*: Induction of anhydrobiosis. In: I. Glazer, P. Richardson, N. Boemare, & F. Coudert (Eds.), *Survival strategies of entomopathogenic nematodes* (pp. 83–98). Luxemburg: EUR 18855 EN Report.

Solomon, A., Solomon, R., Paperna, I., & Glazer, I. (2000). Desiccation stress of entomopathogenic nematodes induces the accumulation of a novel heat–stable–protein. *Parasitology, 121*, 409–416.

Somvanshi, V. S., Koltai, H., & Glazer, I. (2008). Expression of different desiccation tolerance related genes in various species of entomopathogenic nematodes. *Molecular Biochemical Parasitology, 158*, 65–71.

Strauch, O., Oestergaard, J., Hollmer, S., & Ehlers, R.-U. (2004). Genetic improvement of the desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* through selective breeding. *Biological Control, 31*, 218–226.

Stuart, R. J., & Gaugler, R. (1996). Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematode *Steinernema glaseri. Canadian Journal of Zoology, 74*, 164–170.

Surrey, M. R., & Wharton, D. A. (1995). Desiccation survival of the infective larvae of the insect parasitic nematode, *Heterorhabditis zealandica* Poinar. *International Journal of Parasitology, 25*, 749–752.

Tomalak, M. (1989). Improvement of the host-search capability as an objective of selective breeding of *Steinernema bibionis* (p. 36). *Proceedings of Symposium on Biopesticides, Theory and Practice*. Ceske Budejovice, Czech Republic.

Tomalak, M. (1994a). Selective breeding of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) for improved efficacy in control of a mushroom fly, *Lycoriella solani* Winnertz (Diptera: Sciaridae). *Biocontrol Science and Technology, 4*, 187–198.

Tomalak, M. (1994b). Genetic improvement of *Steinernema feltiae* for integrated control of the Western Flower Thrips, *Frankliniella occidentalis. IOBC/WPRS Bulletin, 17*, 17–20.

Tomalak, M. (1994c). Phenotypic and genetic characterization of dumpy infective juvenile mutant in *Steinernema feltiae* (Rhabditida: Steinernematidae). *Fundamental and Applied Nematology, 17*, 485–495.

Tomalak, M. (1997). New morphological variants of infective juveniles associated with mutations in four sex–linked genes of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae). *Fundamental and Applied Nematology, 20*, 541–550.

Tomalak, M., & Mráček, Z. (1998). Scanning electron microscope study on morphological modifications of lateral fields in infective juveniles of mutant *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae). *Fundamental and Applied Nematology, 21*, 89–94.

Tyson, T., Reardon, W., Browne, J. A., & Burnell, A. M. (2007). Gene induction by desiccation stress in the entomopathogenic nematode *Steinernema carpocapsae* reveals parallels with drought tolerance mechanisms in plants. *International Journal of Parasitology, 37*, 763–776.

Tyson, T., O'Mahony–Zamora, Z., Wong, S., Skelton, M., Daly, B., Jones, T. J., et al. (2012). A molecular analysis of desiccation tolerance mechanisms in the anhydrobiotic nematode *Panagrolaimus superbus* using expressed sequenced tags. *BioMed Central (BMC) Research Notes, 5*, 68–92.

Vellai, T., Molnár, A., Lakatos, L., Bánfalvi, Z., Fodor, A., & Sáringer, G. (1999). Transgenic nematodes carrying a cloned stress resistant gene from yeast. In I. Glazer, P. Richardson, N. Boemare, & F. Coudert (Eds.), *Survival of entomopathogenic nematodes* (pp. 105–119). Luxemburg: European Commission Publications.

Wang, X., & Grewal, P. S. (2002). Rapid genetic deterioration of environmental tolerance and reproductive potential of an entomopathogenic nematode during laboratory maintenance. *Biological Control, 23*, 71–78.

Watanabe, M. (2006). Anhydrobiosis in invertebrates. *Applied Entomology and Zoology, 41*, 15–31.

White, E. B., DeBach, P., & Garber, M. J. (1970). Artificial selection for genetic adaptation to temperature extremes in *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae). *Hilgardia, 40*, 161–192.

Wilkes, A. (1942). The influence of selection on the preferendum of a chalcid (*Microplectron fuscipennis* Zett.) and its significance in the biological control of an insect pest. *Proceedings of the Royal Society of London Series B, 130*, 400–415.

Wilkes, A. (1947). The effects of selective breeding on the laboratory propagation of insect parasites. *Proceedings of the Royal Society of London Series B, 134*, 227–245.

Wise, M. J., & Tunnacliffe, A. (2004). POPP the question: What do LEA proteins do? *Trends in Plant Science, 9*, 13–17.

Womersley, C. Z. (1990). Dehydration survival and anhydrobiotic potential. In R. Gaugler & H. K. Kaya (Eds.), *Entomopathogenic nematodes in biological control* (pp. 117–137). Boca Raton, FL: CRC Press.

Yokoyama, T. (1973). The history of sericultural science in relation to industry. In R. F. Smith, T. E. Mittler, & C. N. Smith (Eds.), *History of entomology* (pp. 267–285). Palo Alto, CA: Annual Reviews, Inc.

Zioni (Cohen–Nissan), S., Glazer, I., & Segal, D. (1992). Phenotypic and genetic analysis of a mutant of *Heterorhabditis bacteriophora* strain HP88. *Journal of Nematology, 24*, 359–364.