Source of trait deterioration in entomopathogenic nematodes

*Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during *in vivo* culture

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Summary – The stability of traits important for biological control was studied in the entomopathogenic nematode-bacteria complexes *

*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. Five experimental lines of each species were subcultured for 20 serial passages in *Galleria mellonella* larvae to assess trait stability. Subculturing impaired performance of both *H. bacteriophora* and *S. carpocapsae*. Virulence, heat tolerance and fecundity deteriorated in all *H. bacteriophora* experimental 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 declined for reproductive capacity, whereas virulence declined in two experimental lines. Determination of whether trait deterioration was due to changes in nematode, bacteria, or both symbiotic partners was tested 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 trait, but only the bacterial partner played a role in trait reductions for every trait and species, whereas the nematode was the main source only for *S. carpocapsae* nictation. These results emphasise the important role each symbiotic partner plays in the stability and expression of beneficial traits.

Keywords – bacteria, biological control, heat tolerance, host finding, nematode, nictation, reproductive capacity, virulence.

The success of a biological control agent depends on key traits, particularly compatibility with the target pest, reproductive potential, host-finding ability, environmental tolerance, and ability to culture. Deterioration of traits essential for biological control has been recognised in diverse biological control agents (Hopper et al., 1993). The hymenopteran parasitoid, *Muscidifurax raptor*, deteriorated in longevity and fecundity when cultured in the laboratory (Geden et al., 1992). Another parasitoid, *Trichogramma maidis*, developed deficiencies in host acceptance and suitability (van Bergeijk et al., 1989), whereas phytoseiid mites evolved reduced survival and fecundity (Poe & Ennis, 1970). Microbial control agents have shown similar deterioration of beneficial traits when cultured in the laboratory (Dulmage & Rhodes, 1971; MacKinnon et al., 1974).

Genetic and non-genetic processes may be responsible for trait deterioration in laboratory-cultured biological control agents. Loss of genetic variation due to inbreeding (Hartl & Clark, 1989; Roush, 1990a, b), exposure of deleterious recessive genes and increased homozygosity because of inbreeding (Stuart & Gaugler, 1996), and disproportionate representation of genotypes in successive generations due to genetic drift (Hoy, 1985; Hopper et al., 1993) impair effectiveness of biological control agents when subcultured. Trait deterioration may also result from non-genetic factors such as poor nutrition and diseases (Hopper et al., 1993).

Entomopathogenic nematodes (genera *Heterorhabditis* and *Steinernema*) are attractive alternatives to chemical insecticides because they are highly virulent to many insect pests yet environmentally benign (Kaya & Gaugler, 1993). These nematodes harbour species-specific mutu-
alistic bacteria (*Xenorhabdus* spp. for steiner nematodids and *Photorhabdus* spp. for *heterorhabditids*) that defend against secondary invaders and provide essential nutrients. The development of entomopathogenic nematodes as biological insecticides has resulted in their mass production on an industrial scale (Georgis, 2002), creating selection regimes that favour deterioration of traits relevant for biological control. Several studies have already shown that laboratory culture can lead to deterioration of beneficial traits in entomopathogenic nematodes. Shapiro *et al.* (1996) reported reduced heat tolerance in infective-stage juveniles of *Heterorhabditis bacteriophora* following laboratory rearing. A heat tolerant strain of *H. bacteriophora* isolated from Israel showed loss in tolerance after eight passages through *Galleria mellonella* at 25°C (Shapiro *et al.*, 1996). Other reports include declines in storage stability in *Steinernema carpocapsae* (Gaugler *et al.*, 1989), virulence in *H. bacteriophora* (Grewal & Georgis, 1999), and infectivity and reproductive capacity in *S. glaseri* (Stuart & Gaugler, 1996) following repeated in vivo subculturing.

Although previous studies have shown that entomopathogenic nematodes resemble other biocontrol agents in deterioration of beneficial traits upon subculturing, the underlying mechanisms responsible for deterioration have not been determined and the respective roles of the nematode and bacterial symbionts have not been established. This study assesses deterioration in experimental lines of two nematode-bacterial complexes over 20 passages in insect hosts, permitting a determination of whether the nematodes, their symbiotic bacteria, or both partners, are the source of deterioration on a trait-by-trait basis.

**Materials and methods**

**Cultures**

Soil samples were collected from New Jersey and Georgia, USA, to extract two fresh isolates of *Heterorhabditis bacteriophora* (Hb-NJx and Hb-GA strains), and from New Jersey and Arkansas, USA, to isolate two fresh isolates of *Steinernema carpocapsae* (Sc-NJx and Sc-Cxrd strains) using the *G. mellonella* bait method (Bedding & Akhurst, 1975). To minimise founder effect, ten infected cadavers for each isolate were obtained and emerging nematodes were combined and reared once in *G. mellonella*. A genetically diverse population was created by mixing 10,000 infective juveniles of each isolate of the two nematode species and infecting ten *G. mellonella* at 200 nematodes per host. The resulting mixed population was designated as our base strain. Nematode culturing and experiments were conducted at 25°C except for the heat tolerance bioassay. Infective-stage juveniles were stored (10 days maximum) between passages at 10°C. Last-instar *G. mellonella* were obtained from Northern Bait Co. (Chetek, WI, USA).

**Establishment of experimental lines**

The base populations for *H. bacteriophora* and *S. carpocapsae* were divided into six portions each containing 2000 infective juveniles, one designated as the control line and the others as experimental lines I to V. Change in the control line was reduced by keeping frequency of subculturing and the number of passages to a minimum. Thus, the five experimental lines for each nematode species were reared in *G. mellonella* through 20 passages (two or three generations per passage), whereas the control line was cultured once every fifth pass for four *G. mellonella* passages in total. Each passage of the control line (i.e., at the 5th, 10th, 15th and 20th passage of the experimental lines) was conducted in parallel with the experimental lines just prior to assessment of traits; thus, age of nematodes was not a factor when comparing control and experimental populations. Opportunities for genetic drift were reduced by initiating each subsequent passage with 20 insects exposed to 1000 nematodes; each passage required 16 days from insect infection to nematode harvest. Despite the inability to compare directly the base population (i.e., not subcultured) with the experimental lines due to loss of viability during storage, there are substantial differences in the number of serial passages between the control (four passages) and experimental lines (20 passages) (e.g., see Bai *et al.*, 2005a), permitting parallel comparisons as to changes in heat tolerance, virulence, reproductive capacity, host finding and nictation.

**Heat tolerance**

Heat tolerance was tested using the methods of Shapiro *et al.* (1996). A 0.5 ml suspension of 1000 infective juveniles was added to 5.5 ml tap water in a 10 ml test tube, and the tubes were transferred to a 38°C shaker water bath (100 rpm). After incubation for 8 h, the tubes were held at room temperature for a 12 h recovery period before mortality was determined by probing immobile nematodes. Control and treatments were replicated five times.
HOST FINDING

Nematode response to host volatiles was measured using the quadrant plate bioassay (Grewal & Wright, 1992). Only *H. bacteriophora* was tested because *S. carpocapsae* responds poorly to volatile host cues (Lewis et al., 1992). Briefly, the bases of 9 cm diam. Petri dishes were marked into quarters and then with concentric rings 1, 2 and 3 cm outward from the dish centre. The dish bases were filled with 5 mm of 2% agar, the lids replaced, and the dishes sealed with plastic film. The dish lids had a 3 mm aperture at the outermost edge of one quadrant to accommodate a pipette tip holding a *G. mellonella* larva weighing 200-260 mg. The pipette tip with larva was placed in the aperture for 1.5 h to allow a host volatile gradient to form. One hundred nematodes were then introduced to the centre of the agar surface with a probe through an inoculation port. Nematode location in the assay dish was measured 1 h after inoculation and mean distance travelled towards the host source was calculated. Control and treatments were replicated five times.

NICTATION

Nictation in *S. carpocapsae* was studied using 2% agar plates (9 cm diam.) in which 0.14 g of sand (<150 μm particle size) was scattered over the agar surface to generate a substrate suitable for nictation (Campbell & Gaugler, 1993). One hundred nematodes were transferred to the plate and the proportion nictating (i.e., elevating >95% of their body) was determined after 1 h. Heterorhabditids have not been observed to nictate so *H. bacteriophora* was not included in this bioassay. The control and treatments were replicated five times.

REPRODUCTIVE CAPACITY

Cadavers from the virulence experiments were used to determine reproductive capacity. Four cadavers for each population, comprising one replicate, were placed in a White trap. Emerging nematodes were collected for counting 14, 16 and 18 days post-infection, and reproductive capacity was expressed as infective juveniles produced per mg of insect live weight. Control and treatments were replicated four times. Each replicate used five cadavers resulting in 20 insects exposed per treatment.

VIRULENCE

Virulence was tested in 30 x 30 mm plastic cups holding 35 g of sterile sand (>150 μm particle size) moistened with 1.75 ml of water. A single *G. mellonella* larva was placed in each cup by making a depression in the middle of the sand. The larva was covered with sand and then 100 infective juveniles in 200 μl of water were introduced to the sand surface. Insect mortality was recorded after 72 h. The control and treatments were replicated five times. Each replicate used four cups resulting in 20 insects exposed per treatment.

SOURCE OF DETERIORATION

To determine the source of trait deterioration, that is, whether nematode, bacteria, or both organisms were responsible for deterioration, experiments were conducted by exchanging maximally deteriorated nematodes or bacteria (after 20 passages) with their non-deteriorated (control) symbiotic partners. Thus each species was tested using four nematode-bacteria pairings: i) control (control nematode + control bacteria); ii) nematode only deteriorated (20th passage nematode + control bacteria); iii) bacteria only deteriorated (control nematode + 20th passage bacteria); and iv) both partners deteriorated (20th passage nematode + 20th passage bacteria). The maximally deteriorated lines tested were *H. bacteriophora* experimental line V (all traits), and *S. carpocapsae* experimental lines I (nictation and reproductive capacity), II (virulence), and IV (heat tolerance).

Bacterial cultures for the exchanges were obtained from infected *G. mellonella* haemolymph and plated onto NBTA plates (Dunphy & Webster, 1989). Entomopathogenic nematode eggs, the only stage devoid of bacteria, were obtained by exposing gravid females to an alkaline solution (Lunau et al., 1993). Control and 20th passage nematode eggs were plated onto control and 20th passage bacteria lawns (Gerritsen & Smits, 1993) to create four pairings to test for differences in heat tolerance, reproductive capacity, virulence, host finding, and nictation. Each pairing was cultured on an artificial medium (dog food agar; Kaya & Stock, 1997) until infective juveniles developed, which were used in a single *G. mellonella* passage before testing. Trait recovery, i.e., the degree of improvement in deteriorated traits in the pairings, permitted an assessment of the relative contribution of each symbiotic partner to the observed loss. Control and treatments were replicated five times.
STATISTICAL ANALYSIS

All data were subjected to statistical analysis using Kruskal-Wallis test (Yoshioka, 2000). At the beginning of the experiment (0 passages) and after 5, 10, 15 and 20 passages, treatment effects in each trait assay were tested by using ANOVA, and Tukey’s Multiple Comparison Test was applied to elucidate differences among individual treatment means. Similarly, ANOVA and Tukey’s test were used to determine treatment effects in the source of deterioration assays.

RESULTS

HEAT TOLERANCE

All ten *H. bacteriophora* and *S. carpocapsae* experimental lines deteriorated in heat tolerance when compared to the control at the end of 20th passage (Fig. 1A, B). For *H. bacteriophora*, deterioration was detected as early as the fifth passage in all experimental lines except line II. Experimental line V showed a maximum loss of 33% (*P* < 0.001; *F* = 47.6) (Fig. 1A), which continued at a rate of 2% per passage. Experimental line II remained stable until the 15th passage, showing a loss of 23% in heat tolerance at the end of 20th passage (*P* < 0.001; *F* = 40.79). Loss of tolerance to 38°C ranged from 23% (experimental line II) (*P* < 0.001; *F* = 37.96) to 48% (experimental line V) (*P* < 0.001; *F* = 111.26), showing an average loss of 39 ± 3.0% (mean ± standard error) per experimental line at the end of 20th passage.

*Steinernema carpocapsae* heat tolerance also began to decline from the fifth passage with experimental line IV showing a 39% loss (*P* < 0.001; *F* = 32.81) (Fig. 1B). The experimental line III also deteriorated by the fifth passage (*P* < 0.01; *F* = 28.38) but showed no further reduction. The experimental lines II and V were relatively stable until the fifth passage, deteriorating thereafter at a rate of 4% and 3% per passage. Loss of heat tolerance ranged from 19 and 24% up to 30% in experimental lines I, III, and IV at the end of 20th passage. Overall loss came to 36 ± 3.0% (mean ± standard error) per line when the experiment was terminated.

HOST FINDING

Four of the five *H. bacteriophora* experimental lines showed reduced host-finding ability (Fig. 2A). Losses were first noted from the fifth passage in the experimental line V, with this line suffering overall deterioration after 20 passages of 26% (*P* < 0.01; *F* = 16.04). Experimental lines I and III did not show losses until the tenth passage, declining 30% (*P* < 0.01; *F* = 20.67) and 31% (*P* < 0.001; *F* = 29.92), respectively. Experimental line IV did not deteriorate until the final passage, with a reduction of 17% (*P* < 0.05; *F* = 6.82). The most dramatic deterioration occurred in experimental line I (27%). Notably, the ability of experimental line II to locate hosts did not deteriorate, the only *H. bacteriophora* line in any test which remained stable. Overall, the four deteriorated lines had losses averaging 23 ± 2.2% (mean ± standard error) at the end of 20 passages.

NICTATION

Nictation ability deteriorated steadily in all five *S. carpocapsae* experimental lines (Fig. 2B). Deterioration developed rapidly in some lines, being noted as early as the fifth passage in experimental lines I and IV, declining an average 2% for each additional passage. Deterioration in experimental line III commenced from the tenth passage, and in the experimental line V from the 15th passage. Experimental line I showed maximal (42%) (*P* < 0.001; *F* = 176.42) and experimental line II minimal (19%) (*P* < 0.001; *F* = 32.80) deterioration, with the remaining lines showing intermediate losses of 31-39% at the end of the 20th passage. The overall decline in nictation among all experimental lines averaged 34 ± 4.1% (mean ± standard error).

REPRODUCTIVE CAPACITY

*Heterorhabditis bacteriophora* experimental lines I (22%; *P* < 0.01; *F* = 16.71) and V (15%; *P* < 0.05; *F* = 6.99) showed reduced reproductive capacity (18% loss) within five passages (Fig. 3A) and all experimental lines had reduced reproductive capacity by the tenth passage. When subculuring was completed after 20 passages, all five experimental lines exhibited reduced reproductive capacity, ranging from a loss of 17% per mg of insect live weight for experimental line III to 19-25% for the remaining experimental lines, with an overall average loss of 21 ± 1.3% (*P* < 0.001).

By contrast, *S. carpocapsae* reproductive capacity remained stable until the 15th passage (Fig. 3B), when all experimental lines except III had declined. The remaining four experimental lines deteriorated from 12% (experimental line II; *P* < 0.05; *F* = 6.06) to 22% (experimental line I; *P* < 0.01; *F* = 27.75) with an average loss

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in reproductive capacity of 17 ± 2.0% (mean ± standard error) after 20 passages.

**Virulence**

Most *H. bacteriophora* experimental lines began to display losses in virulence compared to the control at the tenth passage (Fig. 4A) and all lines showed reductions after 20 passages. Virulence losses ranged from 25% (experimental line IV; $P < 0.05; F = 10.00$) to 45% (experimental line V; $P < 0.01; F = 23.14$) and averaged 35 ± 3.5% (mean ± standard error).

Although erratic over the course of the study, virulence was unchanged after 20 passages among three of the five *S. carpocapsae* experimental lines (Fig. 4B). However, the experimental lines II (20% loss; $P < 0.05; F = 6.00$) and V (15% loss; $P < 0.01; F = 16.00$) showed
significant losses in ability to kill insects at the end of 20th passage.

SOURCE OF DETERIORATION; CONTROLS VS BASE POPULATION

Pairing of control nematodes and bacteria did not differ from that of the original base population for any of the five traits tested for either nematode species.

HEAT TOLERANCE

Pairing deteriorated 20th passage bacteria with control nematodes or the converse of pairing deteriorated 20th passage nematodes with control bacteria resulted in infective juveniles displaying heat tolerance comparable to the control nematodes and bacteria pairing for both species (Fig. 5A, B). All three pairings for either species showed greater heat tolerance than the deteriorated nematodes and bacteria pairing ($P < 0.001; F = 38.72$). In short, heat
tolerance of *H. bacteriophora* and *S. carpocapsae* recovered irrespective of whether 20th passage nematodes or bacteria were paired with their respective control partner (*P* < 0.05; *F* = 24.25) (Fig. 5C), suggesting that both partners had contributed to the observed decline of this trait.

**HOST FINDING**

Host finding ability of *H. bacteriophora* recovered when either 20th passage nematodes or bacteria were paired with their respective control partner (*P* < 0.05; *F* = 9.3) (Fig. 5D). Greater recovery
in nictation ability was obtained when control nematodes were combined with deteriorated bacteria \( (P < 0.001; F = 36.25) \). Nevertheless, neither combination resulted in full recovery to the level of nictation obtained in the control nematode and control bacterium pairing. These findings suggest that nematode deterioration was mostly responsible for reduced nictation in our deteriorated experimental lines, with the bacterial symbiont playing an important but lesser role.

**REPRODUCTIVE CAPACITY**

Pairing control nematodes with deteriorated bacteria did not result in recovery of reproductive capacity in *H. bacteriophora* or *S. carpocapsae* complexes \( (P > 0.05; F = 0.13) \) (Fig. 6A, B). By contrast, pairing deteriorated nematodes with control bacteria resulted in reproductive capacity returning to virtually the same level as the control combination \( (P < 0.05; F = 9.8) \). This finding indicates...
that losses in reproductive capacity during subculturing of the two species were due exclusively to changes in the bacteria rather than the nematodes.

**VIRULENCE**

Pairing control nematodes with deteriorated bacteria did not result in significant improvements in virulence for either *H. bacteriophora* or *S. carpocapsae* ($P > 0.05$; $F = 0.13$) (Fig. 6C, D). The pairing of deteriorated nematodes with control bacteria resulted in recovery of virulence for *H. bacteriophora* and *S. carpocapsae* to the same level as the control pairing ($P < 0.05$; $F = 9.8$). This indicated that subculturing had caused changes in virulence of the bacteria and not the nematodes.

**Discussion**

Subculturing impaired the performance of both the *H. bacteriophora* and *S. carpocapsae* nematode-bacteria complexes. Virulence, heat tolerance, and reproductive capacity deteriorated in all *H. bacteriophora* experimental lines, and four of five experimental lines deteriorated in host-finding ability. By comparison, all *S. carpocapsae* experimental lines deteriorated in heat tolerance and nictation, and four of five experimental lines declined in reproductive capacity, whereas two experimental lines showed deterioration in virulence. Moderate differences in the extent and rate of deterioration between the two species were noted but the most important difference was the stability of virulence in *S. carpocapsae*. Arguably the most desirable field trait for a mass-produced biocontrol
agent, Gaugler et al. (1990) also reported stability of virulence when the All strain of *S. carpocapsae* was subcultured for 13 passages in *G. mellonella*. The basis for this stability may lie in the inherent stability of the associated bacterium, *Xenorhabdus nematophilus*, which is the partner primarily responsible for inducing mortality in nearly all host species, including *G. mellonella* (Dowds & Peters, 2002). In a parallel study (Yi Wang, Rutgers University, NJ, USA, pers. comm.) we found *in vitro* subculturing resulted in vast changes in the bacterium, *Photorhabdus luminescens*, associated with *H. bacteriophora*, including alterations in growth, cell size, inclusion body number and size, virulence, and culture colour. By comparison, subculturing *X. nematophilus* did not result in measurable changes to any of the traits tested.

The rate of deterioration was often rapid. Deterioration of the *H. bacteriophora* lines was first noted in the fifth passage for three of the four traits measured, and by the tenth passages all traits showed deterioration in one or more experimental lines. Deterioration in *S. carpocapsae* experimental lines was observed by the fifth passage for heat tolerance, the tenth for nictation and virulence, and the 15th for reproductive capacity. This supports previous reports in the entomopathogenic nematode literature of rapid strain deterioration (Gaugler & Campbell, 1991; Shapiro et al., 1996; Stuart & Gaugler, 1996; Bai et al., 2005a). Wang and Grewal (2002) found reductions
in *H. bacteriophora* stress tolerance, storage stability, and reproduction in as few as three passages. These findings are particularly noteworthy because strains presently mass-produced are subcultured, sometimes for years, before their adoption for commercial use.

Studies on deterioration of entomopathogenic nematode populations invariably focus on the nematode partner and ignore the role of the symbiotic bacteria. Our experiments pairing maximally deteriorated nematodes and bacterial experimental lines with their non-deteriorated symbiotic partner permitted us to examine deterioration independently in each partner, thereby comparing their relative contribution to deterioration for the first time. The source of deterioration varied according to trait, but only the bacterial partner played a role in trait reduction for both species.

Losses of virulence in both nematode-bacterial complexes (including the single deteriorated experimental line for *S. carpocapsae* virulence) were the result of bacterial deterioration during subculturing. This is anticipated given the central role of the bacterial partner in this trait (Dowds & Peters, 2002). The role of the nematode in virulence is not inconsequential; for example, it may aid suppression of the host immune system (Wang & Gaugler, 1998). Insecticidal toxins (Bowen et al., 1998) and enzymes (Dowds & Peters, 2002) produced by the bacteria, however, are unmistakably the principal factors explaining virulence in most insect hosts. Unfortunately, neither the present study nor a companion study (Yi Wang, pers. comm.) measured the biochemical characters associated with virulence. Bai et al. (2005b), however, have observed alterations in *P. luminescens* proteins related to virulence during subculturing and reported that the extent of change increased as the number of culture cycles increased.

The role of the bacteria in reduced *H. bacteriophora* and *S. carpocapsae* reproductive capacity was similarly unsurprising, since the bacteria provide nutrients important for nematode reproduction (Han & Ehlers, 2000). This nutritional relationship is obligate and highly specific for *H. bacteriophora*, as this nematode cannot be cultured under axenic conditions or on other bacteria. *Steinernema* spp. are less fastidious as limited *in vivo* and *in vitro* reproduction occurs in the absence of their symbiotic partner (Han & Ehlers, 2000). A key source for nutrients provided by the bacteria appears to be crystalline inclusion bodies (You et al., 2005), which comprise 40% of total cell protein (Bowen & Einsign, 2001).

Unanticipated was the contribution of the bacterial symbiont to losses in heat tolerance, host finding, and nictation, all traits in which the bacteria played equivalent (heat tolerance and host finding) or secondary (nictation) roles in deterioration compared with their nematode partner. Unlike virulence and reproductive capacity, a direct role in these traits by the bacterial partner is not easily envisioned. The only clear relationship between the bacteria and these traits would be nutritional stress contributing to a general loss of vigour. Entomopathogenic nematode fitness is impacted by nutrition (Abu Hatab et al., 1998), and nictation and host finding are energetically demanding activities requiring sound nutrition. A correlation between reduced host search capacity (e.g., mobility, area searched) and nictation as food reserves are depleted has been demonstrated (Lewis et al., 1995). Despite our suggestion of a nutritional basis for much of the observed deterioration, clearly the underlying cause may be genetic changes in the bacteria. Considering the nematodes were subcultured for 40–60 generations (2–3 generations × 20 *G. mellonella* passages) whereas the bacteria were subcultured for many more generations and have a more rapid mutation rate, genetic change would be predicted to be most liable for the bacterial partners.

Beneficial trait reductions in cultures of biocontrol agents have generally been attributed to genetic changes incurred through founder effect, inadvertent selection, or inbreeding (Van Bergeijk et al., 1989; Geden et al., 1992), although few studies, including the present investigation, have established a specific cause (Hopper et al., 1993). Nematode producers tend to use single isolates from a local parent population, promoting the accumulation of founder effects. Our initial populations were intentionally drawn from large samples collected from geographically distant areas, followed by mixing of the strains, in order to preserve genetic variation and thus diminish drift. Nevertheless, given the restricted number of founding nematodes (Selvan et al., 1993) every infection is a bottleneck event offering the potential for lost alleles. Wang and Grewal (2002) attributed trait deterioration, including loss of reproductive capacity, to inadvertent selection. Reproductive capacity, however, would be expected to be among laboratory-favoured alleles. Inbreeding may have caused trait deterioration in our study. Inbreeding is widely used by breeders to fix desirable traits but genetic erosion results if the limited gene pool caused by subculturing results in the fixation of deleterious alleles (i.e., inbreeding depression via reduced heterozygosity). The genetic source of deterioration cannot be resolved until hybrid crosses are made between the lines and inbreeding indices are established.
One approach to limit deterioration is maintaining genetic diversity through cryopreservation (Bai et al., 2004). Subculturing is still unavoidable because seed cultures are eventually used up and because many generations are needed to move from cryovial to shake flask to final bioreactor. Furthermore, because mortality during cryopreservation can be high (Nugent et al., 1996), genetic bottlenecking may be pronounced, which is why we used a minimally subcultured (four passages) line as our control rather than cryopreservation and reactivation of the base population as in Wang and Grewal (2002). Few entomopathogenic nematode researchers or producers use liquid nitrogen as a storage medium because strains vary in their adaptability to cryopreservation; it is expensive, and mechanical failure or human error can result in complete loss of genetic material. A second approach is to supplement genetic diversity in the laboratory by reintroducing fresh isolates from their source. However, collection of fresh material takes time, may introduce disease, and may be unreliable because it requires the population of the original isolate to persist where it was last found. A third approach to deter beneficial trait loss is to fix traits by creating genetically homozygous inbred lines. Bai et al. (2005a) reported that subculturing of Heterorhabditis bacteriophora (16 passages) resulted in deterioration to virulence, reproductive capacity, heat tolerance, and host finding ability, whereas inbred lines were impervious to change in all beneficial traits. Although some trait deterioration seems inevitable, combining the inbred method, which includes screening to identify superior experimental lines, with cryopreservation to minimise subculturing, offers the greatest potential for preserving beneficial traits.

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References


Deterioration of entomopathogenic nematodes in culture


