

GROUP SELECTION ON POPULATION SIZE AFFECTS LIFE-HISTORY PATTERNS IN THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE*

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Selection is recognized to operate on multiple levels. In disease organisms, selection among hosts is thought to provide an important counterbalance to selection for faster growth within hosts. We performed three experiments, each selecting for a divergence in group size in the entomopathogenic nematode, *Steinernema carpocapsae*. These nematodes infect and kill insect larvae, reproduce inside the host carcass, and emerge as infective juveniles. We imposed selection on group size by selecting among hosts for either high or low numbers of emerging nematodes. Our goal was to determine whether this trait could respond to selection at the group level, and if so, to examine what other traits would evolve as correlated responses. One of the three experiments showed a significant response to group selection. In that experiment, the high-selected treatment consistently produced more emerging nematodes per host than the low-selected treatment. In addition, nematodes were larger and they emerged later from hosts in the low-selected lines. Despite small effective population sizes, the effects of inbreeding were small in this experiment. Thus, selection among hosts can be effective, leading to both a direct evolutionary response at the population level, as well as to correlated responses in populational and individual traits.

KEY WORDS: Artificial selection, group selection, host–parasite interaction, levels of selection, population density, propagule size, *Steinernema carpocapsae*, trade-off, timing of reproduction, virulence, *Xenorhabdus nematophila*.

Group selection is known for its contentious history; yet, it is clear, at least theoretically, that traits can respond to selection operating on multiple levels. The most prominent example of this is altruistic behavior, which, despite being selected against within groups, is predicted to evolve if it is favored by selection among groups (Wilson 1977; Nowak 2006). When looking at traits defined at the level of the individual, it is possible to partition selection acting at the individual level from selection acting at higher levels using a contextual analysis (Heisler and Damuth 1987; Goodnight et al. 1992) or the Price equation (1972). Studies on natural populations employing these approaches have shown that group traits do affect

individual fitness (see Goodnight and Stevens 1997; Table 2), and that this effect can oppose individual-level selection (Tsuji 1995). However, both of these approaches sometimes incorrectly indicate group selection (Okasha 2004) and neither can establish whether there will be an evolutionary response to group selection. Thus, artificial selection at the group level has the advantages that group fitness, and the group-dependence of individual fitness, are strictly defined by the experimenter and that the evolutionary response is measured.

Building on the seminal work of Wade (1977), the majority of laboratory studies on group selection have been conducted

using flour beetles (see Goodnight and Stevens 1997; Table 1). These studies have shown that group selection can cause evolutionary responses in both individual- and population-level traits. Furthermore, they show that, in contrast to individual selection, group selection can be effective on nonadditive genetic variation (Goodnight and Stevens 1997; Wade 2000). Surprisingly, only a handful of studies have been performed in species other than flour beetles; nonetheless, these studies demonstrate the applied importance of understanding group selection. For example, selection among groups of hens based on group egg production not only led to higher egg production, but it also reduced aggression between hens, eliminating the need for the costly practice of beak trimming (Craig and Muir 1996; Muir 1996). In addition, in a study on an RNA virus, Miralles et al. (1997) demonstrated that selection for slower growing virus populations could reduce virulence relative to both random group selection and selection for faster growing populations.

Parasitic infections are one class of phenomena in which a multilevel selection framework is naturally applied (e.g., Levin and Pimentel 1981; Knolle 1989; Bonhoeffer and Nowak 1994; May and Nowak 1995; Mackinnon and Read 1999). Individual parasites within a host compete with each other for access to host resources; thus, within-host selection may favor intense competitive ability or rapid use of host resource. However, this within-host selection may be opposed by among-host selection if increased competition within the host reduces the transmission probability of parasites in that host relative to parasites in other hosts. With this in mind, we imposed artificial group selection to determine whether selection among hosts could cause evolutionary change, and if so, whether other group-level and individual traits would respond as well.

We used the insect-parasitic nematode, *Steinernema carpocapsae*. A multilevel selection approach is especially appropriate in this sexual species, as multiple nematodes must co-infect a host for a successful transmission. Additionally, competition within the host has been shown to affect the number and quality of transmission-stage nematodes leaving the host (Selvan et al. 1993). Finally, these nematodes carry symbiotic bacteria, which could affect the evolution of nematode traits through indirect genetic effects. Previously we have used an experimental evolution approach to determine how differential migration affects nematode reproductive patterns, bacterial interactions, and insect mortality rate (Bashey et al. 2007; Vigneux et al. 2008). Here, we artificially selected on the number of nematodes emerging from a host, a group-level phenomenon akin to population size or group propagule production. Although the number of nematodes emerging from a host is the sum of the individual fecundities, there is also an emergent nature to this aggregate trait, as interactions among individual nematodes and with their bacterial symbionts can influence nematode fecundity (Poinar and Thomas 1966; Han

and Ehlers 2000; Okasha 2006). It is precisely these types of interactions that can lead to different evolutionary responses depending on the level of selection (Wade 2000; Bijma and Wade 2008).

We performed three replicate experiments, selecting among hosts for either a high number or a low number of emerging nematodes. Each experiment consisted of five independent lines of each selection treatment, and was carried out for four episodes of selection. In each episode of selection (hereafter, passage), the fitness of individual nematodes depended on their own reproductive success and on the reproductive success of their group, as only individuals in the selected groups had the potential for non-zero fitness. In the groups selected for high numbers of emerging nematodes, individual selection and group selection should both favor increased fecundity. In contrast, in the groups selected for low numbers of emerging nematodes, we expected that group selection would oppose individual-level selection for increased fecundity. We refer to these treatments as “high selected” and “low selected.” We saw significant responses to selection in one of the three experiments; consequently, we conducted further studies on those experimental lines to determine the role of inbreeding, and to measure any correlated responses to group selection.

Methods

STUDY SYSTEM

The entomopathogenic nematode, *S. carpocapsae*, persists in the soil as a free-living, nonfeeding, and developmentally dormant third-stage juvenile (Poinar and Leutenegger 1968). Each nematode carries its symbiotic bacteria, *Xenorhabdus nematophila*, in a specialized vesicle of the intestine (Bird and Akhurst 1983). Nematodes infect insect larvae through natural openings, and once inside the haemocoel, nematodes resume development and release their bacteria, which reproduce freely inside the insect. Both the nematodes and the bacteria contribute to killing the insect, which occurs within a few days postinfection (Poinar and Thomas 1966; Burman 1982; Dunphy and Webster 1988; Simoes 2000). The gonochoristic (separate sexes) nematodes feed on both host tissue and bacteria, maturing and mating inside the host for one or more generations. Individual nematodes either mature inside the host or leave the host as third-stage juveniles (Wang and Bedding 1996).

There are numerous ways in which selection could act on multiple levels in this system. For example, although an individual nematode benefits by reproducing more, this may reduce the success of the group as a whole, if an individual's greater reproduction is gained by contributing fewer toxins to overcoming the host immune system, or by causing the quality of all juveniles emerging from that host to decline. As nematodes emerging from different hosts may compete against each other in the soil, and

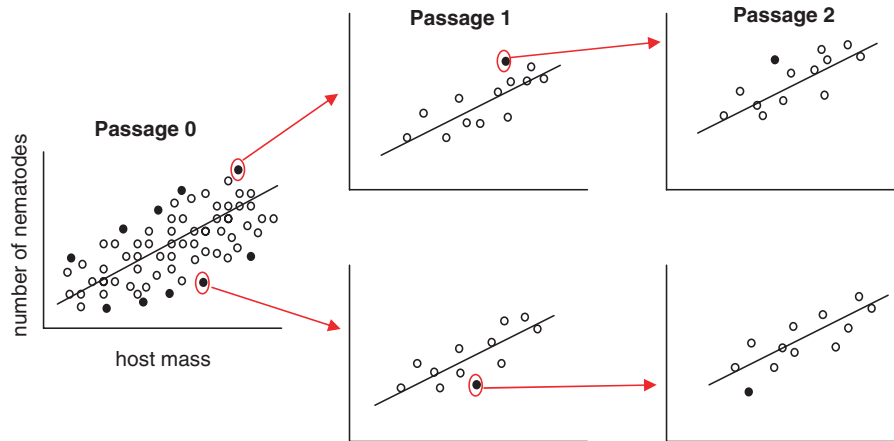


Figure 1. Schematic diagram of the selection protocol. Each experiment was started by infecting 100 insects with approximately 15 nematodes each. The number of nematodes that emerged from each host in this initial passage (passage 0) were counted and regressed against host mass. The five hosts producing the greatest number of nematodes (after accounting for host mass) and the five hosts producing the lowest were selected to start the five high and low lines. Each line was propagated by taking the nematodes that emerged from one host and using them to infect a new batch of 20 hosts. In the schematic, one high line and one low line are shown. In subsequent passages, the regression was performed within each line and treatment and the nematodes from the host with the highest (or lowest) residual value was used to infect a new batch of 20 hosts.

in a new host, the success of an individual may be influenced by these group-dependent factors.

SOURCE POPULATIONS

In this study, we used three of laboratory stocks of *S. carpocapsae*: “U9Gen8,” “U5Gen20,” and “U6Gen21.” These stocks were started from the same initial source population and were propagated identically, albeit independently from each other, for 8–21 passages through larvae of the greater wax moth, *Galleria mellonella*. The initial source population was established with an equal contribution of nematodes from three commercial sources: Integrated Biocontrol Systems Inc. (Greendale, IN: “Sal” Strain), Mellinger’s Inc. (North Lima, OH: an unidentified strain), and Biocontrol Network (Brentwood, TN: “All” strain). Once established, the stocks were maintained in a manner similar to the selection experiment described below with the following exceptions: (1) stocks were outbred by mixing nematodes that emerged from eight hosts prior to infecting new hosts, (2) the infective dose was 200 nematodes per host, and (3) Only nematodes emerging in the first two days of emergence from a host first showing emergence 7–14 days postinfection were given the opportunity to infect new hosts. The insect hosts used for this study (*G. mellonella*) were purchased from reptilefood.com.

SELECTION PROTOCOL

Our goal was to determine whether the number of nematodes emerging from a host was a heritable trait in our laboratory stocks. Thus, we used an experimental design similar to Wade (1977), in that we imposed strong selection, used small initial group sizes,

and propagated groups of nematodes without migration. We performed three replicate experiments (hereafter, Experiments 1, 2, and 3) to determine whether the number of nematodes emerging from a host could evolve in response to direct selection acting at the level of the group. Each experiment was started with a different source stocks (U9Gen8, U5Gen20, and U6Gen21 for Experiments 1, 2, and 3, respectively). These source stocks were randomly chosen to represent our laboratory population. In each experiment, five replicate lines were newly created by selecting for large population size and five replicate lines were newly created by selecting for small population size (Fig. 1). These selection lines were maintained independently of each other for four or more episodes of selection.

At the start of each experiment, 100–200 *G. mellonella* caterpillars were infected individually in 60 × 20 mm petri dishes lined with filter paper (Whatman #1). Each host received a dose of approximately 15 nematodes in 0.5 mL of deionized water. At this dose, approximately 50% of the nematode survived to reproduce (Selvan et al. 1993). Infected hosts were kept at 26°C and were assessed for mortality at approximately 72 h postinfection; at which time, dead hosts were transferred to modified White traps (White 1927). White traps were constructed by placing a 35 × 10 mm petri dish face-down inside a 60 × 20 mm petri dish filled with 15 mL of deionized water and laying a piece of filter paper (55 mm) over the smaller dish. Hosts were placed on the filter paper and monitored daily for nematode emergence from seven to approximately 14 days postinfection. Infective juvenile nematodes were allowed to emerge from these hosts for 4 weeks postinfection in Experiment 1 and 3 weeks in Experiments 2 and

3. The total number of emerged nematodes per host was counted by volumetric subsampling. Hosts that did not produce emerging nematodes during the daily census period were checked an additional time for emergence at the termination of the collection period, but nematodes from these hosts were not counted.

As host mass significantly affects the number of emerging juveniles (Bashey et al. 2007), selection was performed using the residuals of the regression of number of emerging juveniles on host mass (Fig. 1). Five high lines were created by selecting the five highest residuals from successful infections and propagating the nematodes from each separately in 20 new hosts. Similarly, five low lines were established from the five lowest residuals and each propagated in 20 new hosts. These lines were infected and maintained as in the initial passage. In subsequent passages, selection was performed within each line independently by choosing the group with the highest (or lowest) number of nematodes based on line-specific residuals. Experiments 2 and 3 also had five control lines, which were established and maintained in the same manner as the low- and high-selected lines, but the selected group was chosen at random in each passage. Each experiment was maintained as an independent block, with all treatments infecting the same batch of hosts and placed at random within the same environmental chamber. All three experiments were maintained for four episodes of selection. Nematodes were kept in deionized water at 8°C between passages.

CONTINUED RESPONSES TO SELECTION

Experiment 1 was maintained as described above for three further episodes of selection. In addition, in the seventh passage, two new treatments were added: a Low Mix, which was a mixture of the five low-selected lines, and a High Mix, which was a mixture of the five high-selected lines. The aim of adding these treatments was to determine the role of inbreeding in causing the difference between the High and Low treatments. If the Low treatment showed a lower number of emerging nematodes than the High treatment because Low lines suffered more from inbreeding depression, then we would expect a greater relative increase in nematode numbers in the Low Mix treatment than in the High Mix treatment. In each of the mixed treatments, 100 insect larvae were identically infected. These hosts were grouped into five replicates of 20 insects, to match the infection protocol and rearing conditions as closely as possible to the five lines in each of the pure treatments.

In the seventh passage of Experiment 1, several additional traits were measured as well as quantifying the directly select trait, the total number of nematodes emerging per host by 28 days postinfection. First, we examined the timing of nematode emergence by determining the number of nematodes emerging per day during five intervals: days 1–2 postemergence, days 3–7 postemergence, days 8–14 postemergence, day 14 postemergence to day 28 postinfection, and days 29–47 postinfection.

Second, we measured the size of nematodes that emerged in each interval by photographing five nematodes per host for under a compound microscope. For each nematode, length was measured using Image J software (U.S. National Institutes of Health, Bethesda, MD).

To measure the effective population size, an additional 160 hosts were infected with a dose of 15 nematodes from one of two Low lines or from one of two High lines (i.e., 40 hosts per line). Four days postinfection, the hosts were placed in the freezer (–20°C) to halt the infection and preserve the hosts until dissection. Hosts were cut-open, placed in 0.8% saline, and then agitated (100 rpm) for 1 h at 37°C to facilitate the separation of nematodes from the host tissue. Forceps were used to further dissect the hosts and nematode sex was verified under a dissecting microscope. The sex-ratio effective population size was calculated for each host using the standard equation for unequal numbers of male and female (Hartl and Clark 1989).

DATA ANALYSIS

We analyzed the number of nematodes emerging from each host with a repeated-measures analysis of covariance using the Mixed procedure in SAS/STAT software v. 9.1 (SAS Institute Inc., Cary, NC). Experiment (1, 2, or 3), selection treatment (Low or High), and episode of selection (= passage) were considered fixed effects, whereas experimental line (within each treatment) and the interaction between line and episode of selection were considered as random factors. The mass of host was used as a covariate because larger hosts produce more nematodes.

Analyses of the day of first emergence were performed via a Cox proportional hazards regression using the TPHReg procedure in SAS. Host mass was used as a covariate and line effects were accounted for with the covs(aggregate) option. Hosts that emerged after the last daily census were included as censored data. Median and 75% day of emergence were determined by the Lifetest procedure.

Effective population size was nonnormally distributed, thus a Kruskal–Wallis test was performed to see if the treatments or lines differed in effective population size. Probability of host death and the probability of nematode emergence from dead hosts were examined by logistic regression using the Genmod procedure of SAS. In both analyses, treatment (High, Low, High Mix, or Low Mix) were treated as fixed effects and lines within treatments were treated as a repeated factor to account for shared variation among hosts within a line or replicate.

Repeated-measures analyses of variance of nematode length and the number of emerging nematodes per day were performed by treating the experimental line and the insect host as random effects and the treatment and interval as fixed effects. Host mass was used as a covariate in analysis of nematode number, but had no effect on nematode length.

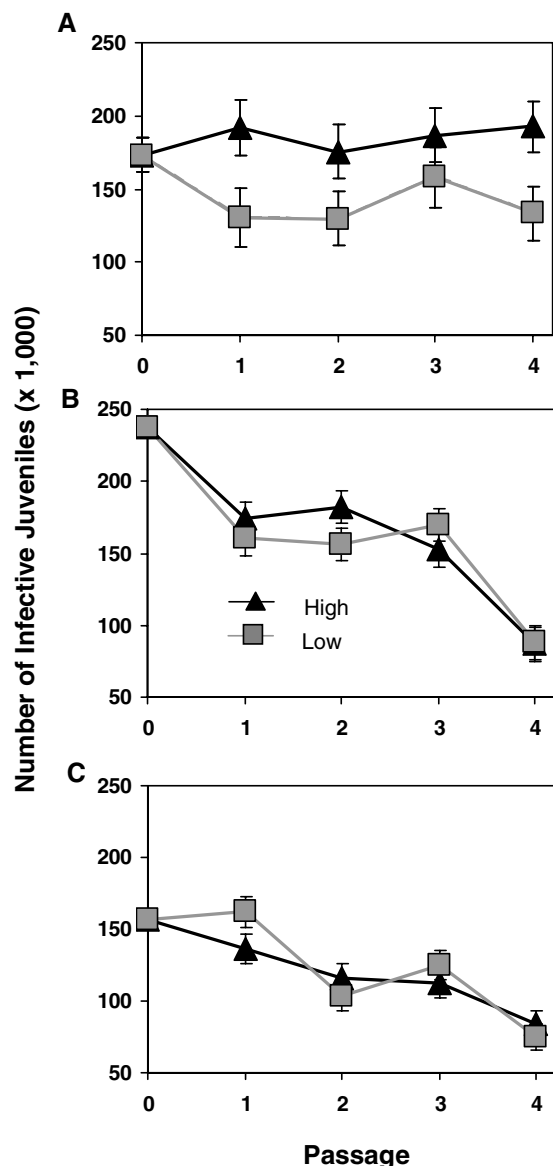


Figure 2. Direct responses to selection on group size. Least square mean number (± 1 SE) of infective juvenile nematodes emerging from a host in the treatments group selected for a high number of emerging nematodes (black triangles) and a low number (gray squares) in (A) Experiment 1, (B) Experiment 2, and (C) Experiment 3. Each symbol represents the average of five lines, each comprised up to 20 hosts per passage. Only Experiment 1 showed a significant difference between the high- and low-selection treatments as reported in Table 1.

Results

INITIAL RESPONSES TO SELECTION

A joint analysis of all three experiments after four passages indicated that the experiments differed in their response to the selection treatments ($F_{2,24} = 5.59$, $P = 0.0102$). Therefore, we proceeded by analyzing each experiment separately to best characterize the response of each experiment. In Experiment 1, a

significantly larger number of nematodes emerged from hosts in the high-selected treatment than in the low-selected treatment (Fig. 2A, Table 1). Moreover, this response was seen immediately after the first round of selection (Fig. 2A) and did not vary with time (treatment \times passage effect in Table 1). As expected, host mass was a significant predictor of number of emerging nematodes (Table 1), and, this relationship did not vary with the selection treatment ($F_{1,366} = 0.04$, $P = 0.8399$).

In Experiment 2, the high-selected treatment resulted in a larger number of emerging nematodes in the first two passages (Fig. 2B); however, this response is not statistically significant unless the data are pooled across lines. Additionally, this effect was not maintained in subsequent passages (Fig. 2B), thus, overall, there was no significant response to selection, nor a significant interaction between treatment and passage (Table 1). Similarly, Experiment 3 showed no significant differences between treatments, nor a significant interaction between treatment and passage (Fig. 2C; Table 1). In both experiments, the number of nematodes produced increased as expected with host mass. The number of nematodes produced also varied significantly with passage number (Table 1). In Experiment 2, this passage effect was due to lower nematode production in the final episode of selection, whereas in Experiment 3, the first and fourth passages were different from the middle two. The control treatments in Experiments 2 and 3 never differed significantly from either selected treatment (results not shown).

We also observed a difference between treatments in Experiment 1 in the day that nematodes were first observed to emerge from their host. In passages 0, 1, and 2, there was a peak of first emergence centered at 10 to 11 days postinfection (with 75% of the hosts showing emergence by day 11), and this timing of emergence did not differ between treatments (Fig. 3A, Table 2). In passage 3, however, there was a difference in emergence time between treatments, with the high-selected treatment showing the “normal” pattern of emergence and the low-selected treatment showing delayed emergence, with only 50% of the hosts showing emergence by day 12 (Fig. 3B; Table 2). Difference in the day of first emergence persisted in passage 4 (Fig. 3C); however, it is only significant if the data are pooled across lines (Table 2). In all passages, nematodes emerged significantly later from larger hosts (results not shown).

SUBSEQUENT RESPONSES TO SELECTION

Consistency of direct response

Experiment 1 was continued for three additional passages. Repeated-measures analysis of all seven passages supports the conclusion that the high-selected treatment produced significantly more nematodes than the low-selected treatment ($F_{1,8} = 6.92$, $P = 0.0301$). Moreover, the magnitude of this difference did not vary significantly over the seven passages ($F_{6,47} = 0.60$, $P = 0.7313$).

Table 1. Direct responses to selection on group size. Fixed effects from repeated-measures analyses of covariance on the number of nematodes emerging from a host are given for each experiment. In each experiment, five replicate lines were established in each high- and low-selection treatment and each episode of group selection coincided with a passage through the insect host. Lines and their interaction with passage were considered as random effects. Only Experiment 1 showed a significant difference between the high- and low-selection treatments. Adjusted treatment means from these analyses are shown in Figure 2.

Fixed effects	Experiment 1		Experiment 2		Experiment 3	
	<i>F</i> -value (df)	<i>P</i>	<i>F</i> -value (df)	<i>P</i>	<i>F</i> -value (df)	<i>P</i>
Treatment	10.01 (1, 8)	0.0133	0.18 (1, 8)	0.6842	0.47 (1, 8)	0.5138
Passage	0.41 (3, 24)	0.7469	32.60 (3, 24)	<0.0001	15.89 (3, 24)	<0.0001
Treatment × passage	0.35 (3, 24)	0.7866	2.15 (3, 24)	0.1201	1.73 (3, 24)	0.1888
Host mass	94.41 (1, 367)	<0.0001	206.92 (1, 540)	<0.0001	49.18 (1, 435)	<0.0001

However, there was significant variation in the number of nematodes produced across time ($F_{6,47} = 11.21$, $P < 0.0001$) with this passage effect due to lower production in passages 5 and 7. As before, more nematodes emerged from larger hosts ($F_{1,638} = 147.66$, $P < 0.0001$).

Inbreeding and effective population size

In passage 7, two additional treatments were added to determine the extent of inbreeding present in our selected lines. These treatments, a Low Mix and a High Mix, combined all five lines from their respective pure treatments. We found no significant difference between the Low Mix and the Low treatment ($F_{1,8} = 0.27$, $P = 0.6149$) or between the High Mix and the High treatment ($F_{1,8} = 0.096$, $P = 0.7619$) in the number of emerging nematodes, indicating that inbreeding depression was not a confounding factor in this experiment (Fig. 4A).

We also measured effective population size (N_e) by infecting and dissecting an additional 160 hosts in passage 7. N_e varied from 2 to 11.67 in hosts that were successfully colonized by both sexes ($n = 104$). N_e did not vary among the four lines tested (Kruskal–Wallis test, chi-square = 4.16, $df = 3$, $P = 0.2447$) or between the high- and low-selection treatments (Kruskal–Wallis test, chi-square = 0.33, $df = 1$, $P = 0.5631$). The median N_e was 3.38; the mean (± 1 SE) was 4.34 ± 0.21 . The sex ratio did not vary among lines or treatments and was significantly female biased (mean percent female = 61.47 ± 2.39 , $n = 139$), as has been reported previously for this species (Lewis and Gaugler 1994).

CORRELATED RESPONSES

Probability of host death and of parasite success

In passage 7, host mortality ranged from 91% to 97%, with the Low Mix having significantly higher mortality than the High Mix (Fig. 4B, $X^2 = 5.20$, $P = 0.0226$, $df = 1$). In contrast, the Low and High treatments showed the same ability to kill their hosts (Fig. 4B). Examination of host mortality rate in earlier passages shows this same pattern of equal mortality rates between the Low and High treatments.

The probability of a successful infection, measured as nematode emergence by day 28, was lower in the Low-Mix treatment than in the High-Mix treatment (Fig. 4C, $X^2 = 4.25$, $P = 0.0392$, $df = 1$). Although the pure Low and High treatments differed in the same way as the mixed treatments, due to the high variation across lines, this difference was not significant (Fig. 4C, $X^2 = 1.31$, $P = 0.2529$, $df = 1$). Examination of the probability of emergence in earlier passages also indicated a trend for lower emergence in Low treatment beginning in passage 3 (Table 2).

Day of first emergence

In passage 7, nematodes from the Low treatment continued to show delayed emergence relative to nematodes from the High treatment (Fig. 3D), with the High treatment showing a peak of emergence at day 9, and the Low treatment showing peak emergence at day 11. In contrast to earlier passages, a large number of hosts showed emergence between days 15 and 28 postinfection, especially in the High treatment. As the actual day of emergence was not recorded for these hosts, we included these data as censored, as was done in our analyses of previous passages (Allison 1995). This analysis of the full dataset indicated a significant time-by-treatment interaction ($X^2 = 5.74$, $P = 0.0166$, $df = 1$), a significant difference between the High and Low treatments ($X^2 = 5.32$, $P = 0.0211$, $df = 1$), and no mass effect ($X^2 = 0.04$, $P = 0.8499$, $df = 1$). After excluding these data, this interaction was no longer significant ($X^2 = 1.78$, $P = 0.1816$). The difference between the High and Low treatments was more pronounced ($X^2 = 6.27$, $P = 0.0123$, $df = 1$), and the expected effect of mass becomes apparent ($X^2 = 5.19$, $P = 0.0227$, $df = 1$). Additionally, there are no significant differences between either Mixed treatment and their respective pure treatments.

Timing of nematode production

The direct response to selection (i.e., the greater number of nematodes emerging by 28 days postinfection in the High vs. Low treatment, Fig. 4A) was driven mainly by a difference in the

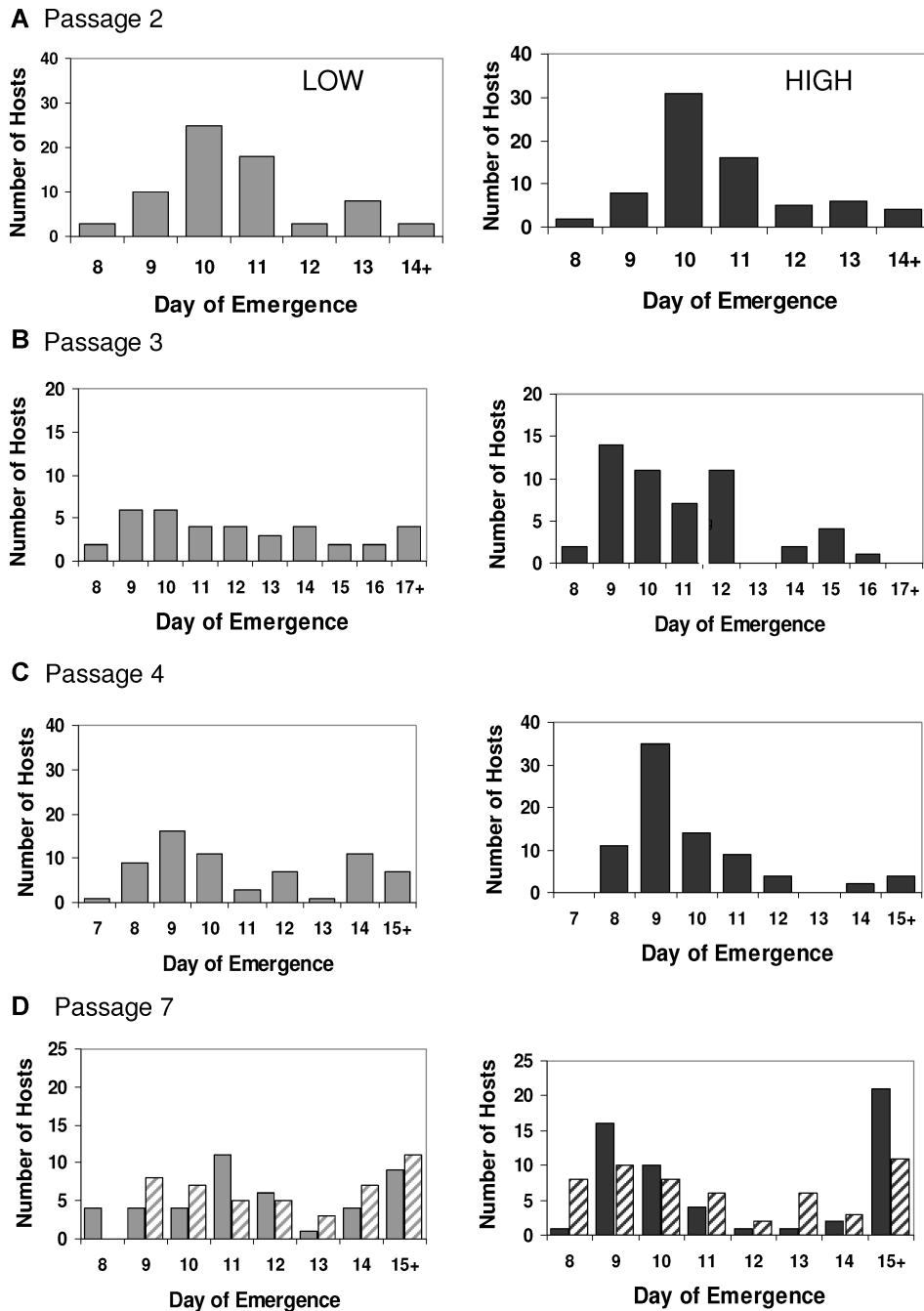


Figure 3. Correlated response in day of emergence to selection on group size. Histograms of the day of first emergence in the low- and high-selected treatments of Experiment 1 after (A) passage 2, (B) passage 3, (C) passage 4, and (D) passage 7. The number of groups (hosts) first showing emergence on a given day is indicated by the height of the bar. The hatched bars in passage 7 indicate the Mixed treatments.

number of nematodes produced between day 3 and 7 postemergence (Fig. 5A). Only during this interval did the Low and High treatments differ significantly in the number of nematodes produced per day ($F_{1,8} = 7.84$, $P = 0.0231$). In contrast, when examining the numbers of nematodes that emerged in the postselection interval (days 29–47 postinfection), the High treatment produced fewer nematodes per day than the Low treatment (Fig. 5A, $F_{1,8} =$

5.61, $P = 0.0455$). These results indicate a trade-off between early and late production as the Low treatment produced more than twice the number of nematodes as the High treatment in this late interval. Nevertheless, this increase was not enough to compensate for their difference prior to day 28, because so few nematodes are produced in the late interval (note the log scale in Fig. 5A). Finally, although the Low treatment never differed significantly

Table 2. Correlated response in day of emergence to selection on group size. Tests for differences in the day that nematodes first emerge from their hosts between treatments selected for high and low numbers of emerging nematodes in Experiment 1. Statistics (df=1) are given from a proportional hazards regression that either accounts for or ignores the lines within each treatment. Product-limit quartile estimates are given for each treatment pooled across lines. Differences significant at the $P < 0.05$ level are indicated in bold.

Passage	N_{low}	N_{high}	Lines not pooled		Lines pooled		Median		75%	
			χ^2	P	χ^2	P	Low	High	Low	High
2	72	73	0.118	0.731	0.195	0.659	10	10	11	11
3	37	52	5.89	0.015	7.13	0.008	12	10	14	12
4	66	79	1.73	0.188	8.53	0.0035	10	9	14	10

from the Low Mix, the High treatment produced significantly fewer nematodes than the High Mix in the last two intervals ($F_{1,8} = 9.95$, $P = 0.0135$), suggesting a degree of inbreeding depression in the High lines.

Nematode size

Nematodes emerging from the Low treatment were significantly larger than nematodes from the High treatment ($F_{1,8} = 22.56$, $P = 0.0014$), suggesting that high nematode numbers were achieved by trading-off nematode size and number. Additionally, nematodes from the High treatment were significantly smaller than nematodes from the High-Mix treatment ($F_{1,8} = 7.40$, $P = 0.0263$), suggesting a degree of inbreeding depression in the High lines.

Discussion

Although group selection is recognized as a potential level of hierarchical selection, its importance as an evolutionary process is still disputed (Bijma and Wade 2008; West et al. 2008; Wilson 2008). In the present study, we found a significant response to selection on population size in one of three experiments. Specifically in Experiment 1, more nematodes emerged per host in lines that were selected for large group size than in lines that were selected for small group size (Fig. 2A). In addition, we saw this response in the first passage after imposing selection, indicating that heritable variation in population size initially existed among groups. Continuation of this experiment for seven episodes of selection showed that this difference remained relatively constant with time, which suggests fast fixation of few alleles of large effect. Furthermore, several traits, involving the timing and size of nematodes produced, evolved as correlated responses to group selection on population size.

The effective population sizes of our founder groups were small (i.e., approximately four of 15 nematodes that initially infected each insect survived to reproductive maturity); nevertheless, the effects of inbreeding depression were minor (Fig. 5). In fact, no inbreeding depression was observed in the selected trait (i.e., group size at 28 days postinfection, see Fig. 4A). Thus, we

are confident that the responses to selection we observed were not caused by greater inbreeding depression in the low-selected lines. Small founding population size was probably important in allowing for an evolutionary response to group selection, as it increased the likelihood of genetic variation among groups.

Correlated responses to selection can exist at both the individual and group level (Goodnight 1989). In our study, after the third episode of selection on population size, we saw a shift in the day that nematodes first began to emerge from their host (Fig. 3). In entomopathogenic nematodes, emergence is a response to both population density and food availability within the host (Popiel 1989). In selecting for lower numbers of emerging nematodes, we most likely lowered the within-host population growth rate, and hence, delayed the cues for emergence. Although this result makes biological sense, the fact that this correlated response did not exist until two passages after the direct response to selection suggests that these two traits are not connected through an unbreakable pleiotropy.

Our selection treatments also resulted in a shift in the decisions individual nematodes make about when and at what size to emerge from the host carcass. Nematodes can either emerge as juveniles or continue developing within the host to potentially reproduce. More nematodes emerged from the High treatment in days 3–7 postemergence, but fewer nematodes emerged later on in the course of the infection (Fig. 5A). Furthermore, nematodes emerging from the High treatment were significantly smaller than those emerging from Low treatment (Fig. 5B). Overall, selecting for a higher total number of nematodes resulted in faster host exploitation and earlier production of nematodes, with the consequences of quicker depletion of host resources and smaller nematode size.

As emerging nematodes must survive without feeding until they encounter a new host, group selection for increased numbers may lower the fitness of individual nematodes. Smaller nematodes may have fewer energy reserves and thus may have lower survival when they are free-living in the soil (Qui and Bedding 2000a,b). Moreover, once exposed to a host, smaller nematodes have been found to be less successful at colonizing and surviving to

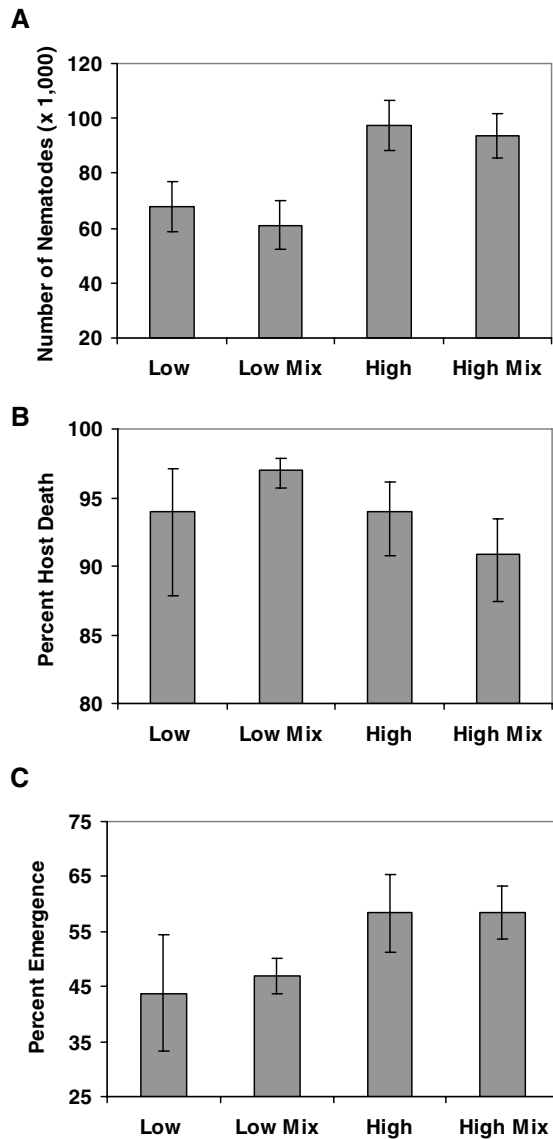


Figure 4. Passage 7. (A) Least-square mean (± 1 SE) number of nematodes emerging per host, (B) Percent (± 1 SE) of infected insect hosts that died, and (C) Percent (± 1 SE) of dead insect hosts that resulted in nematode emergence by 28 days postinfection for each treatment.

reproductive maturity (O'Day and Bashey, unpubl. data). However, in the current study, we saw no differences across treatments in number of nematodes successfully colonizing a host, and we saw a nonsignificant trend toward greater parasite success (i.e., probability of nematode emergence) in the High treatment (Fig. 4C).

The correlated responses we observed in our study were similar to those seen in response to individual-level selection in *Steinernema glaseri* (Stuart et al. 1996). In that study, nematodes were selected based on whether they emerged early or late from their host (i.e., on day 1 vs. after day 7 postemergence). In response, the late lines produced fewer and larger nematodes early

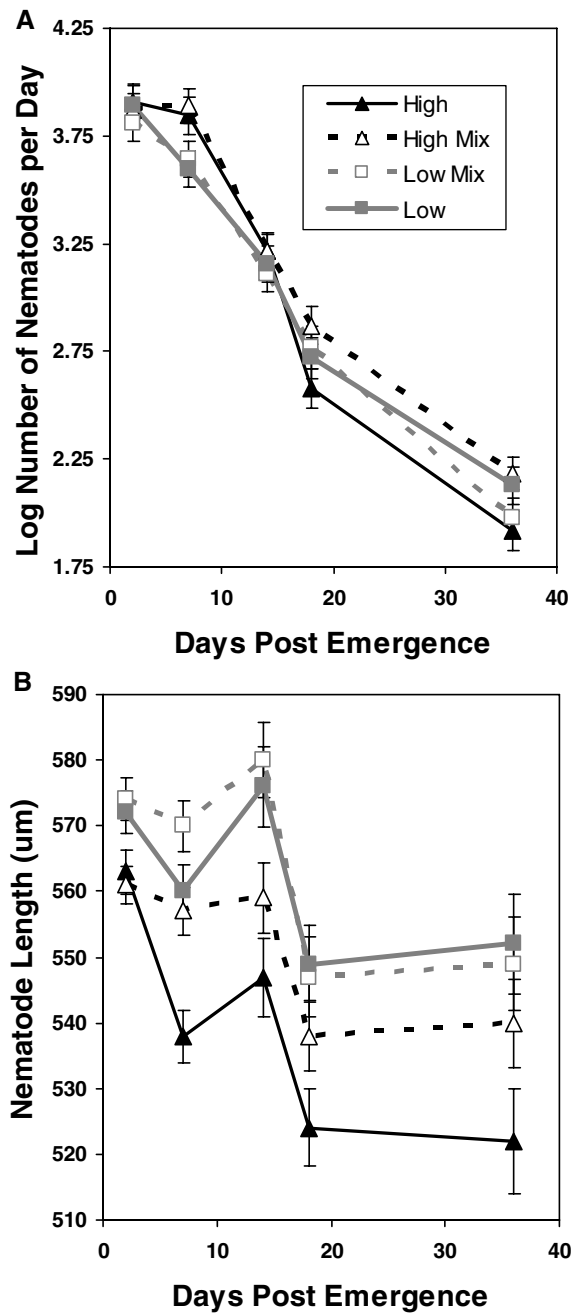


Figure 5. Number and size of nematodes produced per day. Least-square mean (± 1 SE) (A) number of nematodes emerging and (B) length of nematodes emerging in each treatment versus the number of days postemergence. Note nematodes collected over the first four time points contribute to the selected trait, although the nematodes collected on the final time point do not (i.e., these nematodes emerge after the selective cutoff).

in the course of infection. Thus, they found, as we did, that smaller nematode size is correlated with greater early production of nematodes. However, unlike our study, they found no correlations between the timing of nematode production and the total number of nematodes produced, or with the day of first emergence

(Stuart et al. 1996). Although there are, of course, many reasons why these studies could differ in correlations among traits, differences in the level of selection could contribute as well. With group-level selection, evolutionary responses are not restricted by the availability of additive genetic (co)variances, as variation due to interactions among individuals can also respond to selection. Therefore, group selection can be effective where individual-level selection is not, and it can result in different correlated responses (Goodnight and Stevens 1997). Thus, the evolutionary responses to group selection in our study do not necessarily imply additive genetic variances (and covariances) of fecundity, timing of emergence, or size. Our responses could be due to interactions between individual nematodes (Popiel 1989; Selvan et al. 1993; Lewis et al. 2002) or between nematodes and their symbiotic bacteria (Swenson et al. 2000; Sicard et al. 2003).

Finally, given the dramatic responses we observed in Experiment 1, how do we explain the lack of response in Experiments 2 and 3? The explanation may lie in one of the differences between these experiments. First, although the source populations were chosen randomly from our outbred laboratory stocks, the two experiments that showed no response to selection were performed on stocks maintained in the lab for 21 passages, whereas the experiment that did show a response was performed on a stock that had been maintained for only eight passages. Thus, one possibility is that loss of genetic variation occurred in our lab stocks over time. Second, Experiments 2 and 3 differed in that selection occurred 21 days postinfection, whereas in Experiment 1 it occurred 28 days postinfection. Approximately 90% of nematodes emerge from the host within the first 21 days postinfection (Bashey et al. 2007). Moreover, the number of nematodes emerging in days 21 through 28 is comparable to the environmental variation we observe among hosts. Thus, we view it unlikely that the change in the timing of selection affected the response to selection. However, given the complexity of population dynamics within the insect, and the trade-offs we observed, we cannot rule out such a connection.

Alternatively, the different responses to group selection among our three experiments could have been due to the probabilistic nature of generating heritable variation among groups. In addition, because we only propagated one group per line, any genetic variation between groups in the subsequent passages would be limited by the within-group variation of the previously selected group. Strong within-group selection and large environmental variation among groups has been demonstrated to reduce the effectiveness of group selection (Craig 1982; Goodnight 1985; Agrawal et al. 2001). Thus, evolutionary responses to group selection on population size may be a rare finding, because life-history traits are subject to strong selection and are highly phenotypically plastic. In fact, in studies in which population size has evolved in response to group selection, mutational or epistatic variance has

been implicated more than additive genetic variance in causing group-level heritability (Goodnight and Stevens 1997; Miralles et al. 1997; Wade 2000). Furthermore, when no evolutionary response to group selection has been seen, a lack of epistatic variation and large environmental effects have been put forth as explanations. For example, Baer et al. (2000) found no response after six rounds of group selection for increased and decreased population size in a live-bearing fish. Although they considered large founding group size ($n = 44$) and migration among groups as factors contributing to their results, they also suggest a lack of genetically based interactions among individuals and environmental effects might account for their findings.

In summary, in one of three experiments, a 40% difference in population size evolved in response to selection among groups. Thus, despite being an unlikely or weak evolutionary force, when effective, group selection can profoundly change the phenotype (cf. Goodnight and Stevens 1997). Additionally, selection on population size in our experiment affected the timing of nematode emergence and the size of individual nematodes. These results highlight the value of artificial selection, regardless of at what level, for elucidating the functional relationships among traits. Moreover, the results suggest a multilevel selection approach is critical for understanding infection dynamics and the evolution of parasite life histories.

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