Journal of Nematology 36(3):276–280. 2004.

Polymorphic Foraging Behavior Among *Caenorhabditis elegans:* Frequency- and Density-Dependent Selection[1]

John J. Dennehy[2,3] and Todd P. Livdahl[3]

Abstract: Strains of *Caenorhabditis elegans* obtained from their natural soil environment exhibit one of two forms of foraging behavior. Some strains forage solitarily and disperse evenly on a bacterial lawn. Other strains move rapidly until they encounter groups of conspecifics, and then slow their movement and join the group. Strains expressing these behaviors are globally widespread and have been isolated from the same location, suggesting a foraging polymorphism. We hypothesized that density-dependent selection maintains both foraging alleles in populations. Alternatively, both foraging alleles could be retained in populations through frequency-dependent selection. We tested both of these hypotheses by manipulating strain density and frequency, and observing changes in population density over time. Our results indicated that neither density- nor frequency-dependent selection appears to be responsible for the observed polymorphism. The clumping strain consistently out-competed the solitary strain over all treatment levels. We suggest other potential factors that may maintain both alleles in populations.

Key words: Caenorhabditis elegans, density-dependent selection, foraging behavior, frequency-dependent selection, polymorphism.

Natural isolates of *Caenorhabditis elegans* exhibit one of two foraging behaviors (de Bono and Bargmann, 1998; Hodgkin and Doniach, 1997). Individuals of some strains are solitary when feeding (hereafter named solitary strain). These individuals disperse evenly on an agar plate, browse alone, and slow their movement on encountering a bacterial lawn. They react to other individuals by reversing direction and moving away. In other strains, individuals move rapidly on an agar plate until they encounter groups of conspecifics, and then their movement slows and they join the group (hereafter named clumping strain). When isolated, these strains move twice as fast as solitary animals and tend to remain at the border of a bacterial lawn. Figure 1 shows the two strains browsing on bacterial lawns.

A previous study showed that a single nucleotide substitution transformed a solitary strain into a clumping strain (de Bono and Bargmann, 1998). The behavioral differences were attributed to the substitution of phenylalanine for valine in the protein product coded for by the *npr-1* gene. de Bono and Bargmann (1998) determined that this protein product is a transmembrane receptor of the neuropeptide Y (NPY) receptor family. In other organisms, physiological effects of NPY include the regulation of food consumption (Helig and Widerlov, 1995).

The distribution of the two foraging behaviors among natural populations of *C. elegans* nematodes suggests that a foraging polymorphism exists. Of 17 natural isolates sampled by one study, 5 exhibited solitary behavior and 12 accumulated into clumps (Hodgkin and Doniach, 1997). Solitary strains were isolated from England, California, and Wisconsin, whereas clumping strains were found in England, Germany, Australia, Hawaii, California, and British Columbia. In one instance both types were isolated from the same location (Hodgkin and Doniach, 1997). Irrespective of which strain is ancestral, the new isoform may have spread widely from its origin (de Bono and Bargmann, 1998). Alternatively, the transition from social to solitary (or vice versa) may have occurred independently in multiple locations and caused the currently observed global distribution.

The coexistence of two *C. elegans* strains possessing different foraging alleles in the same location implies that either a genetic polymorphism exists or that selective replacement of one form by the other is occurring. The geographical distribution of the two morphs suggests that the former explanation is most likely. Genetic polymorphisms can result from heterozygous advantage (as in the case of sickle cell anemia) or through frequency-, density-, or environmentally dependent selection. Although heterozygous advantage was once believed to be the main cause of polymorphism (Ridley, 1996), Endler (1986) found that only six of hundreds of cases of genetic polymorphisms in the literature could be attributed to heterozygous advantage and thus argued that its importance is likely negligible with respect to other causes of polymorphism. In fact, Clarke (1975, 1979) argued that frequency- and density-dependent selection were the main causes of polymorphisms. Consequently, our initial explorations focused on testing the hypotheses that either density- or frequency-dependent selection maintains the two foraging morphs in populations, rather than attempting to identify a heterozygous advantage or evaluating potential environmental causes of polymorphisms (e.g., spatial or resource variability). Although it may be the case that other ecological or environmental conditions mediate the behavioral differences between the two

Received for publication 1 December 2003.

[1] Funding for this research was provided by grants from the National Institutes of Health and Sigma Xi. Nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).

[2] Department of Ecology and Evolutionary Biology, Osborn Memorial Labs, Yale University, New Haven, CT 06520-8106.

[3] Department of Biology, Clark University, 950 Main St., Worcester, MA 01610.

The authors thank Mario de Bono for providing photographs and technical assistance. Susan Foster and Nicholas Thompson contributed comments that significantly improved the manuscript.

E-mail: jdennehy7@yahoo.com

This paper was edited by S. Patricia Stock.

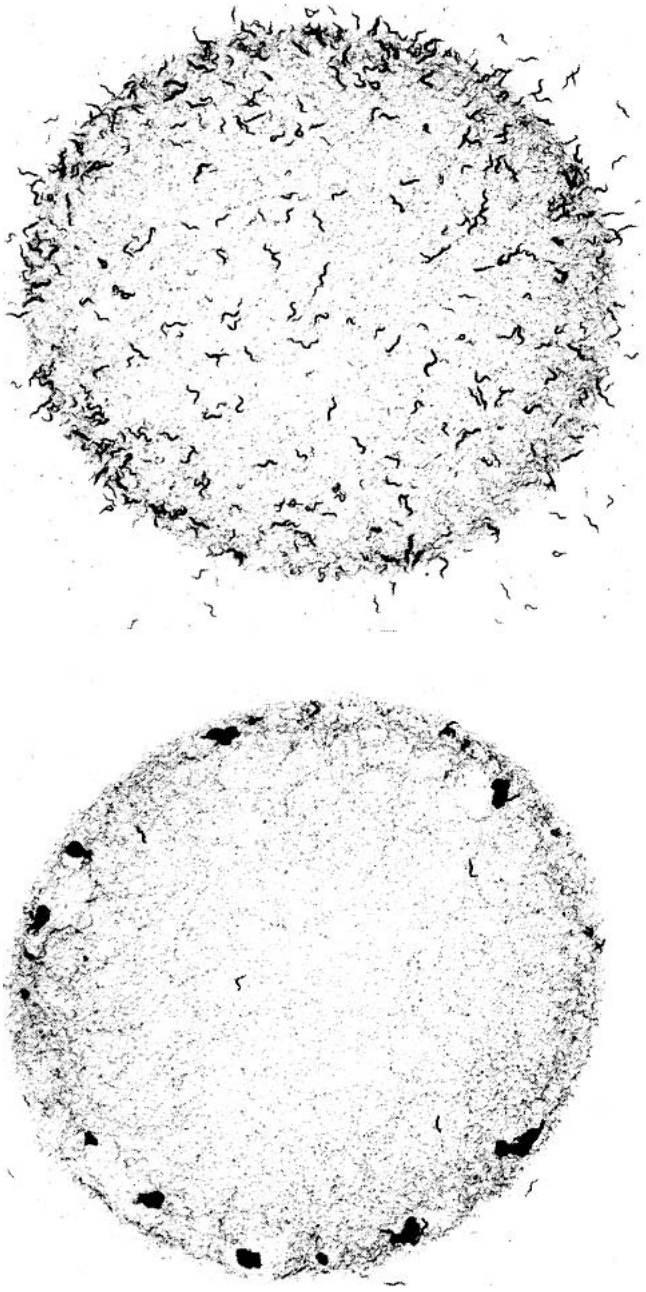

FIG. 1. Solitary (N2) and clumping (CB4932) strains of *Caenorhabditis elegans* as viewed with a dissection microscope at ×25. The top photograph is the solitary strain, and the bottom photograph is the clumping strain. Photographs courtesy of Mario de Bono (Division of Cell Biology, MRC Laboratory of Molecular Biology) and used with permission.

strains, more parsimonious explanations should be explored first.

If density-dependent selection is responsible for maintaining both alleles in the gene pool, then one strain may possess a fitness advantage when nematode population densities are low, and the other strain may be favored when population densities are high. Fluctuating selection would then tend to maintain both alleles in the gene pool. Alternatively, frequency-dependent selection may maintain the two alleles in the population. One allele may possess a fitness advantage when rare but is selected against when abundant. We conducted experiments to determine if density- or frequency-dependent selection maintains both alleles in populations as a behavioral polymorphism.

Methods and Materials

Strains and culture conditions: Two *C. elegans* strains were used in the experiment: PD4792 and CB4932. PD4792 was derived from the wild-type strain N2 but has had the gene for green fluorescent protein (GFP) integrated into its genome (A. Fire, pers. comm.). Like its precursor, N2, PD4792 expresses the solitary phenotype. Since the GFP gene has been inserted directly into the nematode's chromosome, the gene does not lose expression over time. The GFP marker permitted differentiation of the two strains when they were reared together in competition. Green fluorescent protein fluoresces maximally when excited at 400 nm, and living organisms are easily viewed under an epifluorescence microscope. The fitness equivalence of the GFP-integrated strain and its precursor, the N2 strain, was verified by rearing the two strains in competition and tracking each strain's population growth rate. An analysis of the changes in each strain's density in competition over time revealed no differences in their population growth rates ($F = 0.08$, $DF = 19$, $P > 0.05$). This verified that GFP integration had no detrimental effect on PD4972's fitness.

The other strain used in the experiments was CB4932, which was a natural isolate expressing the clumping phenotype (Grewal and Richardson, 1991). Since CB4932 was not observed to produce males (unpublished data), out-crossing between the strains when reared together is likely to be extremely rare. Outcrossing could occur only if a PD4972 male (occurring naturally at 0.2% of the population) mated with a CB4932 hermaphrodite. The effects of this mating on overall strain population growth are small enough to be considered negligible. These facts permit rearing strains together in competition while maintaining the integrity of the GFP marker.

Caenorhabditis elegans populations used in the experiments were maintained at 18 °C on agar seeded with 100 µl *Escherichia coli* (OP50) in Luria broth (L broth) following published methods (Brenner, 1974; Sulston and Hodgkin, 1988). The agar was seeded with *E. coli* 1 day before the onset of the experiments. All organisms were obtained from the *Caenorhabditis* Genetics Center.

Tests for density-dependent interactions: To test the hypothesis that density-dependent selection maintains the two behaviors in wild populations, we varied nematode density while keeping ratio of the two strains constant. Large batches of each strain were obtained by adding 1 ml M9 buffer to each of five culture dishes containing 7-day-old populations (Sulston and Hodgkin, 1988)

and collecting the resulting solutions in 15-ml centrifuge tubes. Subsequently, each batch was cleaned by sucrose flotation (see Comprehensive Protocols Collection, http://cobweb.dartmouth.edu/~ambros/index.html).

Next we created a solution containing equal proportions of each strain. We accomplished this by estimating the density of nematodes in each batch and then diluting the batch with the highest density until the nematode density of the two batches was equivalent. We estimated nematode density of the two batches spectrophotometrically using previously published methods (Patel and McFadden, 1976). The optical density (OD) of each batch was measured 10 times using a spectrophotometer. Student's *t*-test was then used to detect differences in the OD of each batch. The batch with the highest OD was diluted slightly with distilled water. We repeated density measurement until batch OD was statistically indistinguishable. The two batches with equivalent numbers of nematodes were then combined and used as a source material to create the treatments.

We divided the source solution in three equal parts. One part of the combined solution was retained for the high-density treatment. The remaining two parts were diluted with distilled water such that the density of nematodes in the high-density solution was 10-fold greater than the medium density treatment and 100-fold greater than the low-density treatment. We created high, medium, and low-density populations, replicated 12 times, by inoculating 36 petri dishes previously seeded with 100 µl *E. coli* in L broth with 20 µl of the appropriate serial dilution.

For each replicate, the density of nematodes of each strain was estimated on two separate occasions during log phase growth (days 4 and 5 after inoculation). To estimate density of each strain, 1 ml of M9 buffer was added to each replicate. Then 100 µl of the resulting solution was removed and placed on a glass slide. Because the solitary strain is marked with GFP, we were able to distinguish the two strains and estimate their densities by viewing the slide using an epifluorescence microscope. The total density of each strain in the replicate was then extrapolated from the density recorded on the slide. From the resulting data, the population growth rate r was estimated for each strain in competition using the following equation:

$$r = \ln (n_t/n_o)/t$$

where *ln* is the natural log, *t* is time, n_o is initial density, and n_t is subsequent density. The population growth rates for all treatments were compared to determine whether one strain possessed an advantage at certain densities.

Tests for frequency-dependent interactions: To test the hypothesis that frequency-dependent selection maintains the two alleles in the population, the ratio of the two nematode strains was varied while keeping density constant. We obtained large batches of the two nematode strains by washing five culture dishes containing 7-day-old populations with 1 ml M9 buffer and collecting the resulting solution in a 15-ml centrifuge tube. Each batch was then cleaned by sucrose flotation as described. Subsequently, we verified the nematode density of each batch spectrophotometrically as described and made certain that the density of nematodes in the two batches was equivalent. Once batches containing equal densities of each strain were obtained, we removed 0.1, 0.2, and 0.3 ml of fluid from each batch and placed them in labeled centrifuge tubes. The 0.1-ml tubes were combined with the 0.3-ml tubes, and the 0.2-ml tubes were consolidated such that three centrifuge tubes containing 0.4 ml of both strains in solution in frequency ratios of 25:75, 50:50, and 75:25 were produced. Subsequently, we inoculated 30 petri dishes previously seeded with 100 µl of *E. coli* in L broth with 20 µl of solution such that the strain frequency ratios, 25:75, 50:50, and 75:25, were replicated 10 times.

We estimated the density of the two strains of *C. elegans* on each replicate on two separate occasions during log phase growth (4 and 5 days after inoculation) using the methods described above. We then calculated population growth rates for each strain on each replicate. The population growth rates for all treatments were compared to determine whether one strain possessed an advantage at certain frequencies.

Statistical analysis: All analyses were conducted using JMP 4 statistical software (SAS Institute, Inc., Cary, NC). All data met the statistical assumptions of normality, independence, and homogeneity of variances. For the density-dependence experiment, we constructed a full factorial model using population growth rate as the dependent variable, and strain and density as the treatments. A two-way analysis of variance (ANOVA) was conducted to reveal differences between the strain population growth rates with respect to initial density. For the frequency-dependence experiment, we constructed a full factorial model using the population growth rate as the dependent variable, and strain and frequency ratio as the treatments. A two-way ANOVA was conducted to reveal differences between the strain population growth rates with respect to initial frequency. Since negative results were obtained, we performed power calculations at $\alpha = 0.05$ to estimate the probability that our conclusions were correct.

Results

Statistical analysis revealed differences between the population growth rates of the two strains when density was varied ($F = 5.5$, $DF = 71$, $P < 0.0003$) (Fig. 2). However, density had no effect on strain success ($F = 0.36$, $DF = 2$, $P > 0.70$). Rather, the differences in population growth were almost entirely due to strain ($F = 18$, $DF = 1$, $P < 0.0001$). The clumping strain out-competed the

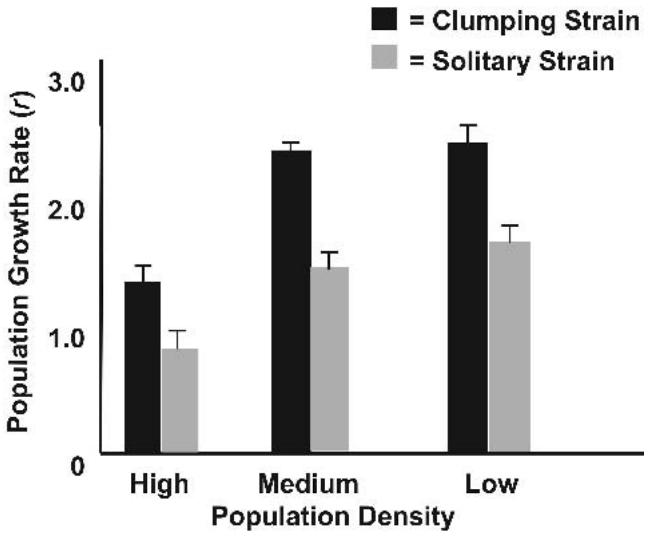

FIG. 2. Population growth rate (r) and its response to population density for the two strains as they were reared in competition. Values are means +/− standard error.

solitary strain over all experimental densities. A power calculation confirmed the robustness of this conclusion ($r > 0.994$).

Since the data indicated that the population growth rates of the two strains were greater at low and medium densities than at high densities, separate one-way ANOVAs were conducted for each density treatment. Furthermore, we tested for differences between the means using the Tukey-Kramer HSD method. None of the population growth means was different from the others.

The mean population growth rates of the two strains were different when frequency was varied ($F = 8.17$, $DF = 59$, $P < 0.0001$) (Fig. 3). However, strain frequency had no effect on strain success ($F = 0.89$, $DF = 2$, $P > 0.42$). Rather, the differences in population growth were almost entirely due to the strain ($F = 17.27$, $DF = 1$, $P < 0.0001$). The clumping strain out-produced the solitary strain over all experimental densities. A power calculation validated the robustness of this conclusion ($r > 0.989$). Observation of the data did not suggest that the population growth rates of the two strains differed between treatments.

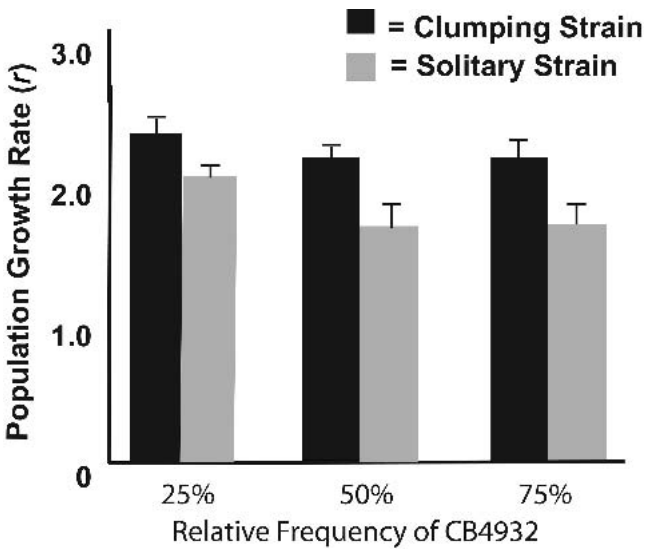

FIG. 3. Population growth rate (r) and its response to the relative frequencies of the two strains as they were reared in competition. Values are means +/− standard error.

DISCUSSION

That both foraging alleles are globally widespread and have been isolated from the same location suggests that both alleles are maintained in populations through a genetic polymorphism. A similar genetic polymorphism for foraging behavior has been reported in *Drosophila* (Osborne et al., 1997; Sokolowski et al., 1997). Larvae homozygous for the rover allele browsed longer on yeast paste and exhibited higher mobility and greater activity than did larvae homozygous for the sitter allele. Density-dependent selection maintained the alternate foraging alleles in populations of *Drosophila*. When larval density was low, sitter strains possessed greater evolutionary fitness than rover strains. By contrast, rover strains had greater fitness when densities were elevated. The results of Sokolowski's (1997) experiments suggest that, when food is abundant, the sitter strains have a selective advantage, and when food is limited, the rover strains are favored by natural selection (Sokolowski et al., 1997).

However, similar results were not obtained in our experiment. By contrast, the results of our density-dependence experiment indicated that the clumping strain out-competed the solitary strain over a wide range of population densities. That is, the clumping strain expressed greater evolutionary fitness than the solitary strain regardless of the density of nematodes in the experimental populations. This result suggests that density-dependent selection does not maintain the two alleles in populations. Given that population densities in the wild may be greater than or less than those used in the experimental treatments, density-dependent interactions may, in fact, exist, but were not detected in this experiment. Prior data suggested that the solitary strain may have an advantage at low densities (Dennehy, unpubl. data). Future investigations will explore that hypothesis.

If either behavior possessed a competitive advantage when rare, then selection would tend to maintain both alleles in the population as an evolutionarily stable strategy (Bell, 1997). However, this experiment indicated that frequency-dependent interactions do not explain the presence of the two alleles in populations. The clumping strain possessed greater fitness at all experimental densities. Unlike the previous experiment, it is unlikely that an interaction would have occurred outside the range of frequencies used. This is because

the lower the frequency threshold where the competitive advantage shifts between morphs, the greater the risk that the allele possessing the lesser advantage will be lost to drift (Bell, 1997). Nevertheless, it may be that frequency-dependent selection exists, but under conditions unrelated to those of the experiment. Perhaps further investigation should focus on strain population dynamics under more natural rearing conditions.

If neither frequency- nor density-dependent interactions maintain the foraging polymorphism in the population, what does? The possibilities include the null hypothesis that the competitive differences reflect selection in action and the competitive displacement of one strain by the other. Alternatively, some unidentified aspect of foraging may provide a competitive advantage to the solitary strain. For example, one strain may be favored when resources are abundant and centrally located, and the other strain may be favored when resources are depleted and patchily distributed.

However, previous experiments showed that neither habitat patchiness, habitat structure, nor resource level mediated the polymorphism (Dennehy, unpubl. data). In the first experiment, the distribution of the bacterial resource was manipulated, but the data showed that the clumping strain was most successful in using both a centrally located resource and a patchy resource. In the second experiment, the population growth rates of both strains were contrasted in a differently structured habitat—a liquid culture. Interestingly, this had the effect of reversing the competitive advantage between the strains, but the difference was not significant. In the final experiment, the two strains were reared over a wide range of resource levels. Although there was some suggestion that the solitary strain was more competitive at the lowest food levels, these differences were not significant. Over the higher resource densities, the clumping strain was consistently superior competitively.

Future investigations would do well to explore other facets of nematode foraging behavior, including the conditions under which clumping and solitary foraging occurs. Perhaps no advantages are available to solitarily foraging nematodes under laboratory conditions, but these advantages may become apparent under more natural conditions.

Literature Cited

Bell, G. 1997. Selection: The mechanism of evolution. NYC: Chapman and Hall.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. Genetics 77:71–94.

Clarke, B. C. 1975. Frequency-dependent and density-dependent natural selection. Pp. 187–200 in F. M. Salzano, ed. The role of natural selection in human evolution. Amsterdam: North Holland.

Clarke, B. C. 1979. The evolution of genetic diversity. Proceedings of the Royal Society of London, Series B, 205:453–474.

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

Endler, J. A. 1986. Natural selection in the wild. Princeton, NJ: Princeton University Press.

Grewal, P. S. and P. N. Richardson, 1991. Effects of *Caenorhabditis elegans* (Nematoda: Rhabditidae) on yield and quality of the cultivated mushroom *Agaricus bisporus*. Annals of Applied Biology 188: 381–394.

Helig, M. and E. Widerlov. 1995. Neurobiology and clinical aspects of neuropeptide Y. Critical Reviews in Neurobiology 9:115–136.

Hodgkin, J. and T. Doniach, 1997. Natural variation and copulatory plug formation in *Caenorhabditis elegans*. Genetics 146:149–164.

Osborne, K. A., A. Robichon, E. Burgess, S. Butland, R. A. Shaw, A. Couldthard, H. S. Pereira, R. J. Greenspan, and M. B. Sokolowski. 1997. Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. Science 277:834–836.

Patel, T. R. and McFadden, B. A. 1976. A simple spectrophotometric method for measurement of nematode populations. Analytical Biochemistry 70:447–453.

Ridley, M. 1996. Evolution, 2nd ed. London: Blackwell Scientific Publications.

Sokolowski, M. B., H. S. Pereira, and K. Hughes. 1997. Evolution of foraging behavior in *Drosophila* by density-dependent selection. Proceedings of the National Academy of Sciences 94:7373–7377.

Sulston, J. E. and J. Hodgkin. 1988. Methods. Pp. 587–606 W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.