Distribution Agreement

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An Empirical Test of Baker's Law:
Dispersion Favors Increased Rates of Self-Fertilization in *Caenorhabditis elegans*

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Abstract

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Baker’s law predicts that population dispersion will select for uniparental reproduction, since dispersed individuals capable of self-fertilization will avoid mate-limitation due to reproductive assurance. However, Baker’s law has been challenging to test empirically because controlled tests are often unfeasible. Here, we test Baker’s law under controlled settings using *C. elegans* and agar plates with three different spatial arrangements of *E. coli* colonies that induce no, low, and high dispersion. Unlike its close dioecious relatives, *C. elegans* appears to rarely outcross in the wild populations, instead primarily reproducing via self-fertilization. Wild populations of *C. elegans* maintain few males, and hermaphroditic *C. elegans* instead fertilize their own eggs. The boom-to-bust ecology of *C. elegans* in conjunction with Baker’s law suggests a possible mechanism for selfing alleles to invade the obligately sexual (outcrossing) ancestor of *C. elegans.* To empirically test the validity of this mechanism and of Baker’s law, we created mixed-mating *C. elegans* populations consisting of 10% wild-type hermaphroditic *C. elegans* and 90% obligately outcrossing *C. elegans.* If selfing is favored, then we expect selfing to increase in frequency and more so in high dispersal treatments if Baker's law prediction is accurate. Both strains were from the same genetic background to mimic the evolution of a spontaneous mutation allowing for self-fertilization. For 18 cycles, the populations were permitted to disperse every two weeks. Self-fertilization invaded all replicate populations across each dispersal treatment. However, increased dispersal selected for greater invasion rates of self-fertilization. Thus, population dispersion can select for the invasion of selfing-alleles into a predominantly outcrossing population, which may explain why *C. elegans* rely on selfing to such an extent. Overall, we provide empirical support for a key prediction of Baker’s law: frequent dispersal favors the evolution of increased rates of self-fertilization

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Introduction

Baker’s law predicts population dispersion will select for uniparental reproduction due to mate limitation. That is, dispersion favors facultative selfing or asexual reproduction, because it allows for reproductive assurance(H. G. Baker 1955). As an extreme example, a solitary selfing individual could colonize a new area whereas an obligately outcrossing individual could not. In mate-limited settings, obligately outcrossing individuals must find and mate with another individual, which could be time and resource intensive and may result in failure to reproduce. Conversely, individuals with the capability to self-fertilize benefit from reproductive assurance and the potential to produce offspring earlier in life than individuals that must have access to mates(Herbert G. Baker 1967). Reproductive assurance conferred by self-compatibility is particularly advantageous in frequently dispersing populations, such as plant species where individuals are sessile and dispersion occurs every generation. However, population dispersion encompasses a wide variety of drastically different ecological scenarios, thus the scope of Baker’s law is restricted to only apply during the expansion phase of colonization—when the population size is relatively low (J. R. Pannell et al. 2015). Afterwards, novel environmental effects may select for biparental reproduction because increased genetic recombination could allow the population to rapidly adapt, if the benefit of outcrossing is sufficient to account for the inherent costs of outcrossing, like the two-fold cost of males or the cost of meiosis (Maynard Smith 1978, 1971; Williams 1975; Charlesworth 1980; Morran, Parmenter, and Phillips 2009; Morran et al. 2011). Beyond the fitness advantage conferred via these inherent costs, selfing individuals could gain a competitive advantage over obligately outcrossing individuals via persistent dispersal. Baker’s law predicts mate-limitation via dispersal drives selection for selfing, since self-compatibility confers reproductive assurance in mate-limited environments (J.

Pannell 1997; H. G. Baker 1955; Herbert G. Baker 1967). Thus, we generally expect selfing to be the predominant form of mating in populations of species that frequently disperse, and particularly after recent dispersal events.

Although Baker's law links two major areas of research in evolutionary ecology (dispersion and mating-system evolution), there remains a lack of conclusive evidence to fully support its predictions (Cheptou 2012). Previous studies on Baker's law provide ambiguous results. Continent-to-island migration is a classic example of long-range dispersion where Baker's law is expected to be applicable. Islands do have lower proportions of self-incompatible taxa, which could indicate that continent-to-island dispersion selects for selfing. However, dioecious flora are overrepresented on several islands, which directly opposes Baker's law (Bawa 2003; Abe 2006; Barrett, Emerson, and Mallet 1996). Furthermore, self-compatibility is more prevalent at the outer ranges of species distributions, where intuitively individuals would have fewer mating opportunities. However, pollen limitation and selfing rates are not necessarily greater on the periphery, meaning Baker's law and mate limitation cannot explain this preference for selfing (Herlihy and Eckert 2005; Stebbins 1957; Busch 2005). Finally, short-lived colonizing ruderal species have confounding life history traits outside of long-range dispersal that select for selfing, which prevents convincing support for Baker's law (Duminil, Hardy, and Petit 2009; Price and Jain 1981). To date, correlational methods of testing such as these have failed to provide clear evidence for or against Baker's law indicating a need for direct empirical tests.

We propose using the androdiecious nematode *Caenorhabditis elegans* as a model organism to directly test the Baker's law prediction that frequent dispersal favors the evolution of increased rates of self-fertilization. The ecology and mating system of *C. elegans* are well-suited to test Baker's law. Despite extensive genetic and developmental studies, the ecology of *C. elegans* is not fully understood. Nonetheless, populations of *C. elegans* appear to undergo a boom-to-bust cycle, where only a small number of individuals can produce hundreds of offspring, resulting in rapid exponential population growth. Wild populations of *C. elegans* are found in decomposing plant materials rich in microbes, their food source (Frézal and Félix 2015; Félix and Braendle 2010). Once depleting their resources, combined overcrowding and starvation signal larval *C. elegans* in the L2 stage to enter an alternative, very mobile, stress-resistant life-stage called dauer that allows them to survive for months instead of the typical 2-week *C. elegans* life span (Cassada and Russell 1975; James W. Golden and Riddle 1984; J. W. Golden and Riddle 1982). Behavioral adaptations in dauer *C. elegans* enable them to disperse in search of a new food source; upon colonizing a bacterial patch they mature, reproduce, and the cycle begins anew (Félix and Braendle 2010). Similar to many plants, *C. elegans* frequently disperse, often to previously uncolonized areas; thus, Baker's Law may explain why natural populations of *C. elegans* are primarily hermaphroditic with relatively rare males and infrequent outcrossing events. The *fog-2* gene can control the development of sperm in hermaphroditic *C. elegans*(Schedl and Kimble 1988). Hermaphroditic *C. elegans* with a *fog-2(wt)* allele, hereafter referred to as the "mixed-mating allele", reproduce through self-fertilization and by outcrossing with males. Although hermaphrodites have both sperm and eggs, they cannot outcross with other hermaphrodites as the hermaphrodite/female morphology lacks a male tail to deliver sperm(Baldi, Cho, and Ellis 2009; L'Hernault et al. 1997; Schedl et al. 1997). An autosomal recessive loss-of-function allele, *fog-2(q71)*, hereafter referred to as the "obligately-outcrossing allele", prevents sperm production in homozygous hermaphrodites by inhibiting the downregulation of feminizing effects of *tra-2*, a sex determination factor (Clifford et al. 2000).

Thus, hermaphrodites homozygous for *fog-2(q71),* essentially females, must outcross with males to reproduce and are obligately outcrossing. Sperm production in males is not affected by *fog-2* mutations, so all female or hermaphroditic *C. elegans* are capable of mating with males regardless of the male's genotype at the *fog-2* locus(Anderson, Morran, and Phillips 2010). Due to the manipulable mating-system, boom-to-bust population cycle, and short life spans, *C. elegans* is an ideal system to test Baker's law empirically.

Here, we utilized *Caenorhabditis elegans* as a model organism to test a critical prediction of Baker's law. By spatially arranging *E. coli* colonies on agar plates, we induced three different levels of dispersion under laboratory conditions: high, low, and none. To mimic natural dispersion conditions, we induced dauer via starvation between dispersals. We simulated the spontaneous mutation of the mixed-mating allele into a population fixed for the obligately-outcrossing allele by introducing a small proportion of wild-type hermaphroditic *C. elegans* homozygous for the mixed-mating allele into an obligately outcrossing population with the same genetic background pending some variation in the obligately outcrossing population. We observed how selfing-rates change in these mixed-mating populations during repeated dispersals to environments to which they had been previously exposed. By forcing the population*s* to disperse to the same environment that they have been previously adapted, we limited confounding ecological effects and focus only on selection due to dispersion. By only altering the level of dispersion between treatments, significant differences between treatments' selfing rates can be attributed to the effects of dispersion. Thus, we hypothesized that while invasion will occur in all treatments invasion rates will increase with dispersion.

Methods

Caenorhabditis elegans strains were established as described in Slowinski *et. al* 2016. In brief, a wildtype strain CF3 (CF3 WT) was made from EMS mutagenesis of a systemically inbred strain of CB4856 (PX382) and 30 generations of passage under laboratory control conditions. Then, the obligate outcrossing allele, fog-2(q71), which contains a nonsense mutation G→A that prevents sperm production, was backcrossed into CF3 to create an obligately outcrossing CF3 strain (CF3 OO). Dauer populations were created by incubating nematodes at 20°C for 2 weeks on sterile 10 cm Petri dishes with 24 ml of autoclaved NGM lite (US Biological, Swampscott, MA). To measure invasion from within the population, only the CF3 WT and CF3 OO strains were used from Slowinski *et. al* 2016, since they are from the same genetic background despite having some genetic variation.

Spatial Arrangement of E. coli colonies

Dispersion was induced on agar plates by manipulating the spatial arrangement of OP50 *E. coli* colonies (the food source) on a 10 cm Petri dish with 24 ml of autoclaved NGM lite. The bacteria were cultured in Lysogeny broth (LB) and incubated at 28°C for 24 hours at which point they reached confluency. Plates were seeded by pipetting 10 µl of confluent LB culture to each colony location. The spatial arrangement of the colonies is described in Figure 1; colonies are spaced 1, 5, and 50 mm apart from each other and 0, 35, and 35 mm from the center of plate. Increased distance between colonies resulted in decreased movement between those colonies. Therefore, different colony spacing patterns were used to create the null, low, and high dispersion treatments respectively (fig. 1)**.** The plates were then incubated at 28°C for 24 hours.

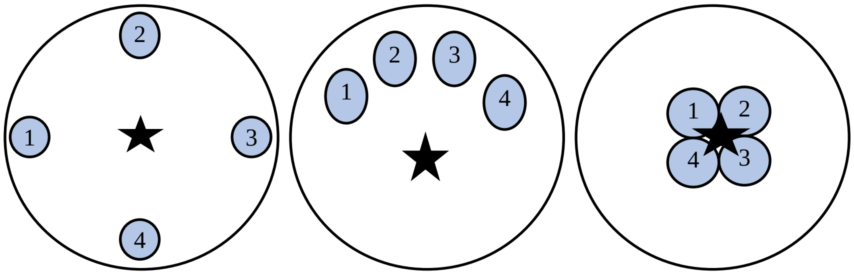

Figure 1. Left to right, these arrangements correspond to no (1 mm between colonies, 0 mm from star), low (5 mm, 35 mm), and high dispersion (50 mm, 35 mm). The four *E. coli* colonies used as a food source for the *C. elegans* are marked by blue numbered circles. Stars mark where dauer *C. elegans* are placed.

Movement Assay

To demonstrate that the high and low dispersion treatments correspond to different levels of dispersion, the movement rates between the colonies were measured. Initially, 20 GFP *C. elegans* (mixed mating JK2735) were liquid transferred in M9 buffer directly onto colony 1, while 20 mixed mating CF3 *C. elegans* were liquid transferred directly onto all other colonies. A third treatment was implemented where the GFP *C. elegans* were transferred to colony 2 to observe the differences between the inner and outer colonies. All treatments were replicated 3 times for a total of 9 populations of approximately 80 nematodes. This assay was performed on a mixed group of *C. elegans* adults and L4 larvae. Each population was incubated at 20°C for the duration of the experiment. At 24 hours, the number of GFP and CF3 nematodes in each colony was counted to calculate the rate of movement between colonies. We used the nonparametric Kruskal-Wallis ranked sum to test for treatment differences (high versus low dispersion) in the number of individuals that moved to a different bacterial colony within 24 hours. The analysis was performed in JMP 12 (SAS Institute, Cary, NC).

Dauer Distribution

To ensure dauer *C. elegans* would distribute equally to the four colonies in each dispersion arrangement (no, low, and high) and not disproportionally aggregate in one colony, 200 mixed mating CF3 (PX382) *C. elegans* were liquid transferred directly onto the center of the treatment plates (stars in fig. 1) in replicates of 10 for a total of 30 populations. Each population was incubated at 20°C for the duration of the experiment. The number of dauer nematodes in two random colonies chosen by a pseudo-random number generator was counted at 12 hours. We then calculated the differences between the two colonies within each replicate. We tested the effect of treatment (no dispersion, low dispersion, and high dispersion) on the difference between the number the individuals at two colonies using a Kruskal-Wallis ranked sums test in JMP 12.

Mixed-Mating Invasion into Obligately Outcrossing Populations

Initially, the obligately outcrossing (CF3 OO) and mixed-mating (CF3 WT) CF3 *C. elegans* were starved for 2 weeks to generate dauer *C. elegans* mimicking the natural boom-to-bust life cycle of *C. elegans*. Then, approximately 180 obligately outcrossing and 20 mixed-mating dauer *C. elegans* were liquid transferred in M9 buffer to the center of the Petri dish for each treatment in replicates of 12 for a total of 36 populations that were about 10% mixed-mating. After populations incubated at 20°C for 24 hours, the male frequency in a random colony chosen by a pseudo-random number generator (https://www.random.org/) from each Petri dish was observed by counting the number of male and female/hermaphrodite nematodes. The chosen colony (nematodes and *E. coli* included) was then transferred to a sterile NGM lite Petri dish (the starvation plate).

The populations on the starvation plates were incubated at 20°C for 15 days (about 3 generations) at which point most of the nematodes were in dauer. Then approximately 200 nematodes from each population were liquid transferred to the dispersal plates. Relatively constant transfer population sizes were maintained by counting the number of individual nematodes in three random 10 µl samples of our nematodes/buffer liquid transfer solutions and adjusting the volume of solution transferred to new dispersal plates as described in Slowinski 2016. Each population was then incubated at 20°C for 24 hours, and a random colony was transferred to a starvation plate after determining the male frequency in that colony. This cycle was repeated 15 times for each population.

Outcrossing rates were calculated from male frequency data by subtracting the frequency of males produce from non-disjunction of the X chromosome from the male frequency and multiplying by 2 (Stewart and Phillips 2002); in cases where the outcrossing rate exceeded one or was less than zero, the outcrossing rate became one or zero respectively. Selfing rates were calculated as one minus the outcrossing rate (Stewart and Phillips 2002; Slowinski et al. 2016). Mean selfing rate data conformed to assumptions of normality and equal variance at generations 6, 12, and 15, as determined by Shapiro-Wilk and Levene's tests in JMP 12 (SAS Institute, Cary, NC). We performed separate ANOVAs in JMP 12 and tested the main effect of dispersion treatment (no dispersion, low dispersion, high dispersion, and no dauer exposure) on the mean selfing rate of replicate populations at generations 6 and 12 of experimental evolution. Additionally, we performed an ANOVA on the mean selfing rate values from generation 15 of experimental evolution, but only tested the effects of the treatments that were exposed to the dauer life stage (no dispersion, low dispersion, and high dispersion). We conducted contrast tests between treatments using Student's t-test in JMP 12.

Results

Movement and Distribution

There was significantly more movement between colonies in the low dispersion treatment than the high dispersion treatment. That is, when CF3 *C. elegans* were placed in one of the four colonies, significantly more CF3 *C. elegans* migrated to a new colony in the two low dispersion treatments than in the high dispersion treatment ($\chi^2_1 = 6.11$, P = 0.047) (Figure 2A). The no dispersion arrangement controls for movement, since the *C. elegans* do not need to move to find food. Additionally, there was no significant difference in how dauer *C. elegans* dispersed to the four colonies between the three treatments. Specifically, when dauer *C. elegans* were placed in the center of the treatment plates, the mean variation between the colony population counts was not significantly different between any of the three treatments 12 hours after plating ($\chi^2_1 = 2.54$, P = 0.28) (fig. 2B). Thus, the high dispersion treatment forms more isolated communities than the low dispersion treatment confirming that the high dispersion arrangement does induce a greater level of dispersion.

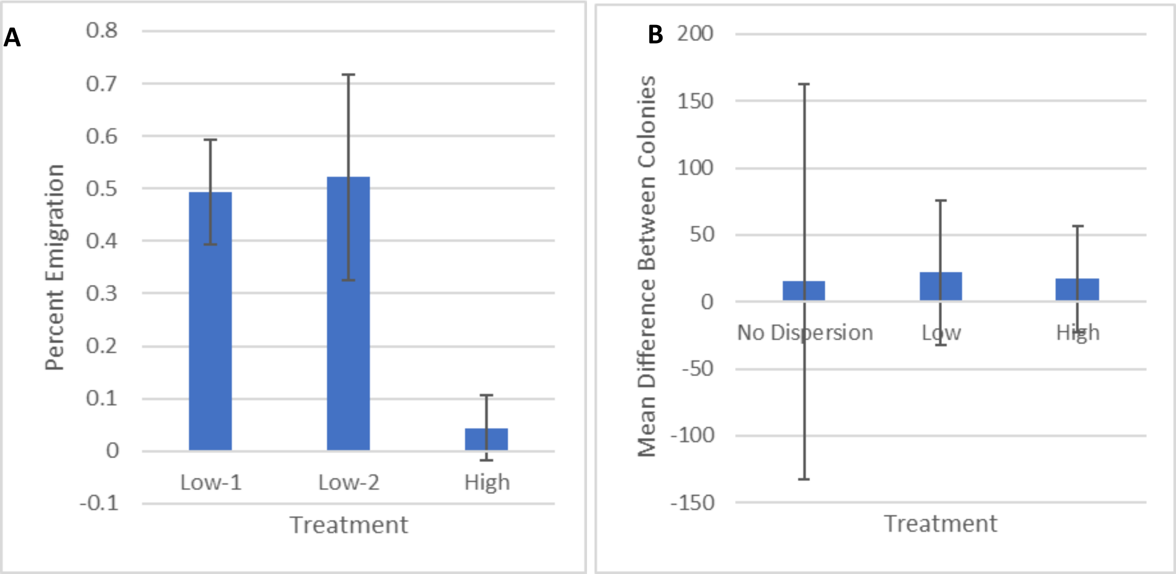

Figure 2. *Left,* The mean difference between the population sizes at the two counted colonies with each spatial arrangement replicated 10 times is shown 12 hours after placing 200 dauer CF3 WT *C. elegans* in the center of the plate. There is no significant difference between the three treatments (P = 0.28). Error bars represent plus and minus one standard error. *Right***,** The percent of the CF3 WT *C. elegans* population that moved to a colony other than their starting colony was

calculated 24 hours after placing 20 adult CF3 WT *C. elegans* in one colony and 20 GFP *C. elegans* in the other three colonies. Error bars represent plus and minus one standard error.

Selfing Invasion

After six generations of experimental evolution, the mean selfing rate in the high dispersion treatment was significantly higher than selfing rate in the low and no dispersion treatments (table 1; Student's t-test, $P < 0.05$). However, the low dispersion treatment and no dispersion treatment did not exhibit significantly different selfing rates after 6 generations of passage (table 1; Student's t-test, $P > 0.05$). The selfing rate in the high dispersion treatment was again significantly greater than the low and no dispersion treatment after 12 and 15 generations of experimental evolution (Generation 12: table 2; Student's t-test, $P < 0.05$; Generation 15: table 3; Student's t-test, $P < 0.05$). The low dispersion treatment populations briefly exhibited greater selfing rates than the no dispersal in generation 12 (table 2; Student's t-test, $P < 0.05$), however this difference was not maintained throughout the remainder of the experiment (Generation 15: table 3; Student's t-test, $P > 0.05$). This indicates that greater rates of dispersion select for greater selection for selfing and the mixed-mating allele (fig. 3).

Table 1. ANOVA Statistical Results for Selfing Invasion at Generation 6

Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**P**
Treatment	0.46	3	0.154	3.72	0.0182
Error	1.77	43	0.041		
Total	2.23	46			

Table 2. ANOVA Statistical Results for Selfing Invasion at Generation 12

Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**P**
Treatment	2.25	3	0.751	35.27	<0.0001
Error	0.92	43	0.021		

Total	3.17	46

Table 3. ANOVA Statistical Results for Selfing Invasion at Generation 15

Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**P**
Treatment	0.23	2	0.115	6.09	0.0057
Error	0.60	32	0.019		
Total	0.83	34			

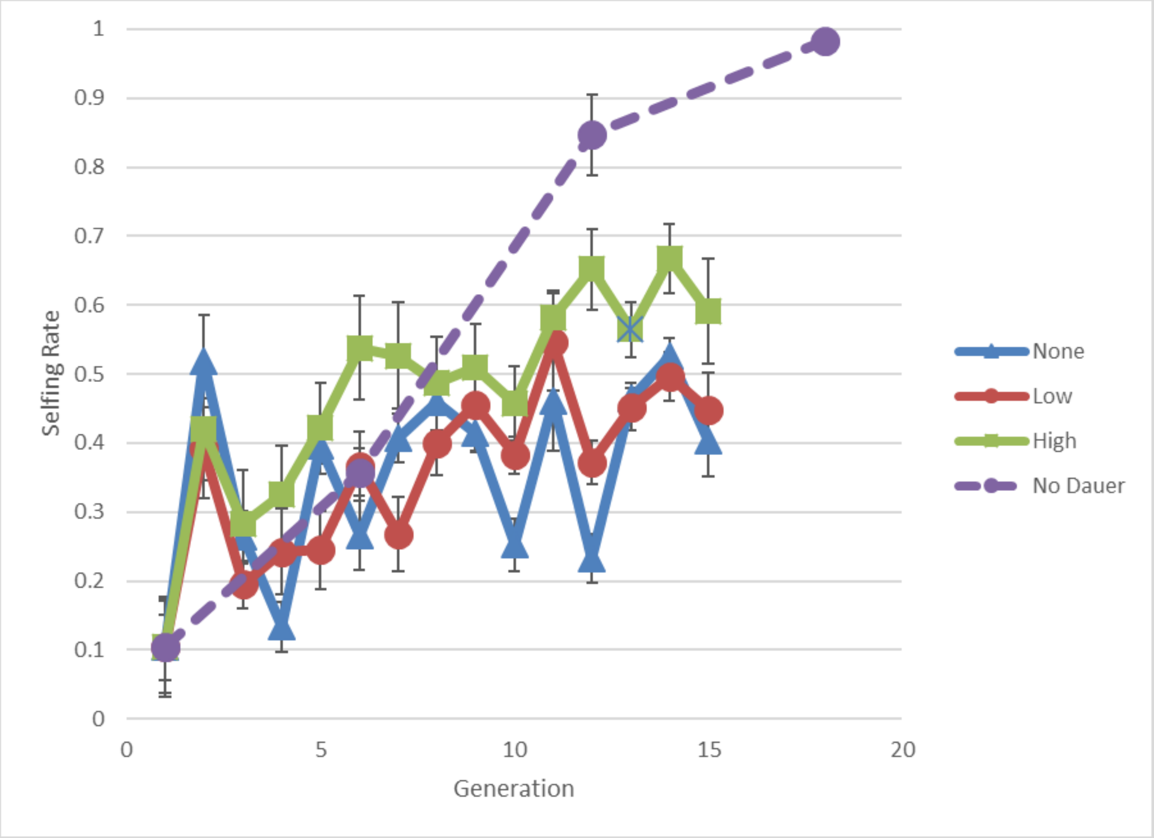

Figure 3. Selfing rates at each generation were counted in one pseudo-randomly chosen colony per replicate with 12 replicates per treatment. The selfing rate in the three dauer treatments was calculated every generation for 15 generations, while the selfing rate in the "No dauer" (dashed purple line) treatment was calculated every 6 generations for 18 generations. The selfing rate in the high treatment (solid green line with square markers) was consistently greater than the low treatment (solid red line with circle markers), which was not consistently greater or less than the no dispersion treatment (solid blue line with triangle markers). Thus, forcing dauer hindered selfing invasion, while increased dispersion selected for greater selfing rates.

The high dispersion treatment selfing rate was significantly greater than the selfing rate in the no-dauer treatment at generation 6 (table 1; Student's t-test, $P < 0.05$), but the no-dauer selfing rate was greater than the high dispersion selfing rate at generation 12 (table 2; Student's t-test, $P < 0.05$). By generation 18, the no-dauer treatment mean selfing rate approached the maximum value of 1, meaning the mixed-mating allele was at or near fixation. Conversely, the means of the populations experiencing dauer conditions in the dispersion treatments did not climb above 70% selfing. Thus, inducing dauer between dispersions hindered selfing invasion compared to the non-dauer treatment.

Overall, selfing invaded at least partially across all treatments. The high dispersion treatment had a consistently higher selfing rate than the low and no dispersion treatments. There was no consistent trend between the low and no dispersion treatments. Initially, the high dispersion treatment had a greater selfing rate than the no dauer treatment, yet the no dauer treatment quickly became almost entirely selfing whereas forcing dauer prevented full selfing invasion in the other treatments.

Discussion

To empirically test Baker's law, we experimentally evolved predominantly obligately outcrossing *C. elegans* populations, harboring lineages capable of self-fertilization, under conditions requiring different degrees of dispersion. We measured the selfing rate in populations over time to determine the rate at which selfing lineages invaded the obligately outcrossing populations. Baker's law predicts that dispersion can select for uniparental reproduction over biparental reproduction(H. G. Baker 1955; Herbert G. Baker 1967). In the context of our experiment this means populations experiencing greater rates of dispersion should permit the selfing lineages to invade at a greater rate than populations that experience less dispersion. Therefore, the high dispersion treatment should select for increased selfing rates. As predicted, we found that selfing rates in the high dispersion treatment were significantly greater than the low and no dispersion treatments. However, there was no consistent trend between the low and no dispersion treatments. There is no quantitative metric for dispersion, so the degree of dispersion in our treatments is relative. Thus, the lack of difference between the low and no dispersion treatments may be due to an insufficient amount of dispersion in the low dispersion treatment to observe a significant effect. Overall, our results provide empirical support for Baker's law, since greater dispersion selected for selfing as indicated by the selfing allele invading more in the high dispersion treatment.

The selfing allele invaded all populations to some extent as expected since the selfing allele invaded obligately outcrossing *C. elegans* populations in Slowinski *et al.* 2016 in the absence of pathogens(Slowinski et al. 2016). In static environments, the cost of males gives selfing hermaphrodites a competitive advantage of outcrossing *C. elegans*(Maynard Smith 1978, 1971). Although invasion occurred in all treatments, the no-dauer treatment eventually reached fixation of the selfing allele whereas all dauer treatments maintained outcrossing. This difference indicates that the dauer state promotes male maintenance and outcrossing, which is expected because male *C. elegans* are more likely to survive the dauer state and dauer increases the propensity to outcross in both hermaphrodite and male *C. elegans* with the CB4856 background, the same genetic background used in this study(Morran et al. 2009). Increased outcrossing and male frequency due to persistent dauer exposure may be why the selfing allele only partially invaded in the dauer treatments. The degree of selfing invasion may be greater in other strains, since not all strains exhibit these dauer tendencies and maintain very low male frequencies despite frequently entering dauer. Ultimately, that the selfing allele partially invaded in the CB4856 background despite the effects of dauer provides greater evidence of increased dispersion selecting for selfing.

However, dispersion enhances the degree of invasion, yet we did not test if mate limitation is the driving force behind the apparent increased selection for selfing in the high dispersion treatment. Measuring the time-to-egg-laying between treatments or similar experiments would be necessary to assess the extent of mate-limitation that occurred. The lack of movement in the high dispersion treatment suggests mate limitation could be a possible factor as the stochastic nature of dispersion would occasionally produce colonies with unbalanced sex ratios. Outcrossing hermaphrodite *C. elegans* must find and mate with a male, where as self-compatible *C. elegans* could immediately begin producing offspring once reaching adulthood. Mate limitation induced by unbalanced sex ratios would only serve to exacerbate the delay outcrossing *C. elegans* would face compared to selfing individuals. By reproducing faster, self-compatible individuals would gain a competitive advantage that would be enhanced by dispersion thus serving as a possible mechanism of Baker's Law.

Our experimental set up falls short of mimicking long-distance dispersion, such as island-continent dispersal, where gene-flow between populations would be extremely rare. Even in the high dispersion treatment, some movement between colonies occurred. Increased dispersion and isolation between colonies should only serve to strengthen our results. Furthermore, some mixing between colonies is more likely to be indicative of *C. elegans* dispersion in nature and so provides insights into the evolution of *C. elegans* mating system. Similar to our experimental set up, self-compatibility in the genus *Caenorhabditis* evolved at least twice from an obligately outcrossing ancestor (Kiontke et al. 2004; Haag and Doty 2005; Nayak, Goree, and Schedl 2004). Even though outcrossing has evolved or been maintained in *C. elegans* under some experimental conditions, like the presence of co-evolving pathogens, since dispersion selected for selfing invasion in our study, frequent dispersion may explain the high propensity for selfing seen in wild *C. elegans* populations(Slowinski et al. 2016; Morran et al. 2011).

Overall, our results provide empirical evidence that increased dispersion can select for uniparental reproduction over biparental reproduction, thus supporting Baker's law. However, the strength of selection favoring selfing is likely sensitive to environmental factors that influence mate limitation and the extent of dispersal. Further, factors beyond dispersion that indirectly select for outcrossing or self-incompatibility, like the dauer state, may also attenuate the effects of Baker’s law. Thus, the parameters and life history of the dispersing population may strongly affect the context in which Baker’s law applies. Furthermore, we have only demonstrated that dispersion selects for selfing when dispersing to a familiar environment, thus selfing may not be selected for when dispersing to novel environments. Rapid adaptation to novel environments can favor outcrossing over selfing, which may negate the effects of Baker’s law, even under conditions of frequent dispersal (Morran, Parmenter, and Phillips 2009; Morran et al. 2011; Slowinski et al. 2016). Despite using an animal model, our results generally support Baker’s law, which can be applied to any frequently dispersing population, including plants and other sessile species. If sessile individuals disperse to an area without a means to outcross, (for example when a flowering plant disperses outside the range of its pollinator), they will be inaccessible to all mates, adding another component of mate limitation besides isolation(J. Pannell 1997). Since dispersion and reproduction are intimately connected in sessile species, the effects of Baker’s Law may be even stronger in those communities.

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