LETTERS

The effects of competition and predation on diversification in a model adaptive radiation

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Much of life's diversity is thought to have arisen through successive rounds of adaptive radiation—the rapid diversification of a lineage into a range of ecologically and phenotypically distinct species[1–3]. Both resource competition and predation have been suggested as mechanisms driving this process[4,5], although the former is better studied than the latter[6,7]. Here we show experimentally how predation by a protist, *Tetrahymena thermophila*, affects diversification in a model adaptive radiation of the bacterial prey, *Pseudomonas fluorescens*. We estimate the frequency-dependent fitness functions of competing niche-specialist prey in the presence and absence of predation, and use these to test hypotheses about the extent (measured as the number of new genotypes) and rate of diversification. Competition and predation independently generated diversifying selection that we show is capable of driving prey diversification to similar extents but at different rates, diversification being markedly delayed in the presence of predators. The cause of this delay stems from weaker diversifying selection due to the reduction in prey density caused by predation. Our results suggest that predation may play an under-appreciated role in driving adaptive radiations.

The rapid diversification characteristic of adaptive radiation is thought to be driven by strong divergent natural selection generated through resource competition in the presence of abundant ecological opportunity (defined as vacant niche space)[4]. The impact of predation on the extent and rate of diversification in prey remains controversial[6]. Comparative[8,9] and experimental[10] evidence clearly implicates a role for predation in generating novel ecological opportunities through the evolution of predator-resistance strategies or access to predator-free space. The effect of predation on the strength of diversifying selection, and so both the extent of phenotypic divergence and the rate of diversification, is less clear. Predation may interact synergistically with competition to promote diversification if, for example, phenotypically intermediate types are susceptible to predation[11,12]. Alternatively, diversification may be slowed, or halted altogether, if predators reduce prey densities sufficiently to prevent resource competition[13].

To understand the manifold effects of predation on diversification, we estimated the strength of diversifying selection due to competition, predation and their combination by measuring the frequency-dependent fitness functions of two of the most common niche-specialist genotypes observed during the model adaptive radiation of *Pseudomonas fluorescens* SBW25 in spatially structured (static) microcosms. The two genotypes are the ancestral broth-colonizing 'smooth' and the biofilm-forming 'wrinkly spreader'. Wrinkly spreader, which occupies the air–broth interface of a static microcosm, is invariably the first niche-specialist to arise by mutation during diversification from the ancestral smooth[14] and in the absence of predation both types are stably maintained by negative frequency-dependent selection in static microcosms[15]. The slope of the line relating wrinkly spreader relative fitness to its initial frequency gives an estimate of the strength of frequency-dependent selection; a negative slope with an x-intercept between zero and one indicates that selection is divergent, each type being fittest when rare. The slope of this relationship in the absence and presence of predators provides estimates of the strength of divergent selection due to competition and competition in combination with predation, respectively. We obtained estimates of the effect of predation independently of competition by adding an antibiotic to the medium at a concentration that prevents bacterial growth without killing the cells and to which the predator is resistant (see Methods).

Our results, shown in Fig. 1, reveal two notable effects of predation on frequency-dependent selection. First, fitness is always negatively frequency-dependent (slope ± s.e. tested using a two-tailed, one-way t-test against a null hypothesis of zero slope; competition alone: -8.74 ± 0.95, $t = -9.22$, d.f. = 53, $P < 0.0001$; predation alone: -4.66 ± 0.69, $t = -6.79$, d.f. = 57, $P < 0.0001$; competition and predation: -3.35 ± 0.96, $t = -3.47$, d.f. = 48, $P < 0.0001$), suggesting the operation of diversifying natural selection in both the presence and absence of predation. Second, predation reduces the strength of frequency-dependent selection, a result confirmed by the significance of the interaction between the starting frequency of

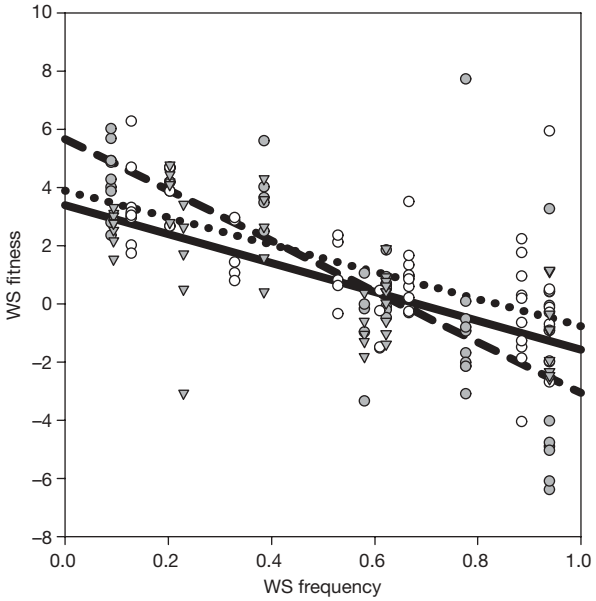

Figure 1 | Frequency-dependent fitness functions due to competition (dashed line and filled circles), predation (dotted line and open circles) and their interaction (solid line and triangles). Fitness is measured as the selection rate per day (r), defined as the difference in the malthusian parameters between wrinkly spreader (WS) and smooth genotypes. Frequency is an estimate of initial wrinkly spreader frequency.

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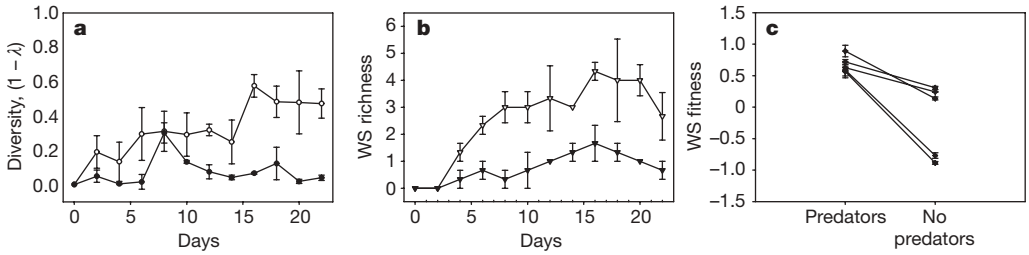

Figure 2 | The effect of predation on the diversification of wrinkly spreaders in minimal media. **a**, Diversity, measured as the complement of Simpson's index ($1-\lambda$), is greater in the presence of predators (open circles) than in their absence (filled circles). **b**, The increased diversity is due to the emergence of additional wrinkly spreader genotypes, WS richness, with predators (open triangles) than without predators (filled triangles). **c**, Five phenotypically distinct wrinkly spreader genotypes had increased fitness in the presence of predators compared to that in their absence. Error bars, ± 1 s.e.m. ($n = 3$).

wrinkly spreader and treatment in an analysis of covariance ($F_{2,158} = 10.33$, $P < 0.0001$).

The observation of negative frequency-dependent fitness between smooth and wrinkly spreader in the absence of predators is consistent with previous results[15], and probably stems from the intense competition for limiting resources, especially oxygen, caused by the combination of spatial structure and population growth. Wrinkly spreader has a fitness advantage when rare because it forms a self-supporting mat that colonizes the air–liquid interface and permits access to oxygen. Fitness declines as it becomes more common, both because resources are relatively more abundant in the broth and because the mat becomes heavier, eventually sinking under its own weight[15,16]. In the presence of *T. thermophila*, who are generalist filter-feeders, selection remains negatively frequency-dependent, as before, but its strength is weakened, probably because resource competition is weakened by the approximately tenfold reduction in prey density due to predation (bacterial cell density per ml $\pm$ 1 s.e. as follows: without predators, $(2.86 \pm 0.65) \times 10^9$; with predators, $(2.78 \pm 0.79) \times 10^8$; ANOVA: $F_{1,10} = 38.22$, $P < 0.0001$). Fitness remains frequency-dependent because the mat provides a refuge from predators for wrinkly spreaders when rare (as in ref. 17). We suspect that wrinkly-spreader fitness declines when common in the presence of predators, however, because *T. thermophila* preferentially feeds at the surface where oxygen is more abundant, an observation we have confirmed by contrasting the abundance of *T. thermophila* just below the air–broth interface in static microcosms with and without mineral oil on the surface, which acts to restrict the diffusion of oxygen into the media (mean density of *T. thermophila* per ml $\pm$ s.e. as follows: with oil, 8,888 $\pm$ 320; no oil, 2,777 $\pm$ 320: two-sample *t*-test, $t_4 = 8.61$, $P = 0.0007$; see Methods).

The negative frequency-dependent fitness between smooth and wrinkly spreader that we have observed is a hallmark of diversifying selection associated with adaptive radiation[18]. Moreover, the observation that predation generates diversifying selection independently of competition suggests that predators should be capable of driving diversification in *P. fluorescens* under conditions where resource competition cannot. To test this prediction, we cultured replicate populations of initially isogenic *P. fluorescens* in the presence and absence of predators in static microcosms containing a minimal salts medium with glucose as the sole carbon source. Previous work has shown that the characteristic *P. fluorescens* adaptive radiation does not occur under these conditions, although wrinkly spreaders occasionally arise at low frequency[19]. Our results indicate that diversification was more extensive in the presence of predators than in their absence (Fig. 2a), and that this was largely owing to the emergence of novel wrinkly spreader genotypes (Fig. 2b) resistant to predation (Fig. 2c; analysis of variance contrasting fitness in the presence and absence of predators for five independent wrinkly spreaders isolated from microcosms containing predators: $F = 34.57$, $P < 0.0001$, d.f. = 27). These results suggest that predation alone is sufficient to drive adaptive radiation through the creation of ecological opportunity afforded by resistance.

Predators also decreased the strength of frequency-dependent selection, suggesting that a second effect of predation on adaptive radiation may be to slow the rate of diversification. To test this prediction, we followed the progress of diversification in the presence and absence of the predator by destructively sampling static KB (King's B medium) microcosms inoculated with the smooth ancestor intensively over the course of eight days. The results, shown in Fig. 3, are striking. The dynamics of diversification resemble the characteristic logistic pattern expected from theory[20–22], the major difference between treatments being the timing at which diversity arises: average richness among replicates becomes significantly greater than one after 48 h in the absence of predators (one-way *t*-test: $t = 3.21$, d.f. = 4, $P = 0.0163$) and 64 h in their presence (one-way *t*-test: $t = 2.45$, d.f. = 4, $P = 0.035$). This result is consistent with those from simple models of the dynamics of gene frequencies under different strengths of frequency-dependent selection (see Supplementary Information). Given the large prey population sizes (approximately 10^6–10^7 cells ml^{-1} in the presence of predators after 24 h in this experiment), this delay in diversification is unlikely to be caused by changes in the supply of novel genotypes through mutation. We also observed a slower maximal rate of increase in richness in the presence of predators, though this difference was not significant ($b \pm 95\%$ confidence limits (CL): no predators, 0.102 ± 0.010 for time = 32–104 h; predators, 0.088 ± 0.017 for time = 48–104 h). We could not detect a difference in the average maximum diversity (mean $\pm$ 95% CL between 112 and 200 h in both cases: no predators, 6.24 ± 0.32; predators, 5.50 ± 0.49). Parameter estimates obtained from fitting a logistic growth model having a breakpoint and incorporating a time lag before diversification begins lend further support to these results (see Fig. 3 and Supplementary Information).

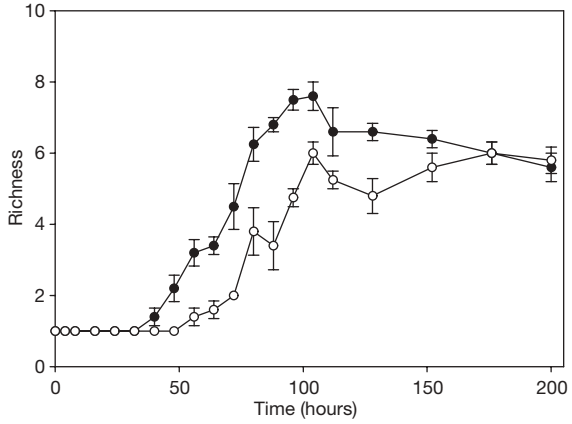

Figure 3 | Dynamics of diversification in the presence and absence of predators. Each circle represents the mean richness of five replicate microcosms, for microcosms with predators (open circles) and without predators (filled circles). Error bars, ± 1 s.e.m.

Our results suggest that predators can modulate the progress of an adaptive radiation in at least two ways. First, predation—by itself or in concert with competition—may generate diversifying selection by creating novel ecological opportunities in the form of access to predator-free space. This suggestion has been made previously[6], and experimental evidence exists to support a role for predation in driving phenotypic divergence among prey[10] and the *de novo* evolution of a single predator-resistant type[23,24]. However, ours is the first direct experimental evidence that predation spurs diversification of prey into a range of predator-resistant phenotypes characteristic of adaptive radiation. Our work also highlights a phenomenon not previously observed: the effect of predation on diversification is most pronounced when the underlying range of resources, and so opportunities for ecological specialization, are limited. Note also that a comparable experiment[13] to ours observed the opposite effect: a bacteriophage predator imposed strong directional selection for resistance, leading to the emergence of a single resistant type whose phenotype differed among microcosms owing to the stochastic effects of low prey population densities caused by infection.

Second, and perhaps less intuitively, predation may slow or delay diversification when the range of available resources is sufficiently large to generate abundant vacant niche space. The underlying cause of this effect stems from changes to the strength of diversifying selection mediated by the combination of resource competition and the selective pressure imposed by predation. Slower rates of diversification due to predation have not previously been observed and may explain, in combination with the greater ecological opportunity afforded by islands, why diversification rates on islands may often be faster than on continents[25].

These results may also help explain why the most spectacular radiations seem to be driven primarily by competition and not predation[26,27]. It seems reasonable to suggest that the range of ecological opportunities offered by resource availability at the lowest trophic levels will often be larger than those generated solely by predation. If so, then predation may only be observed to cause adaptive radiation in exceptionally stringent circumstances, namely, when there are few opportunities for resource specialization. That being said, our observation that predation can spur diversification to similar phenotypic end-points as competition underscores the difficulties faced in teasing apart these two mechanisms in natural systems. The primacy of resource competition in adaptive radiation may be more apparent than real, reflecting the practical challenges associated with conducting experiments capable of disentangling the effects of competition from predation[6] rather than any real bias in the causes of diversification in nature. Either way, our work suggests that predation may play an important—and perhaps under-appreciated—role in explaining the spatially and temporally episodic nature of diversification during the history of life[28].

METHODS

Frequency-dependent fitness experiments. We estimated the fitness of a wrinkly-spreader (WS) genotype chosen at random from a diverse community of *P. fluorescens* that evolved in a KB microcosm, in competition against the ancestral smooth (SM) morph across a range of seven starting frequencies. Fitness was calculated as the selection rate constant r using the equation[29]: $r = (\ln[WS_{final}/WS_{initial}] - \ln[SM_{final}/SM_{initial}])/\text{time}$, where the subscripts final and initial refer to the densities of cells (per ml) of each genotype at the end or beginning of the experiment, respectively. The slope of the regression of fitness against starting frequency gives an estimate of the strength of frequency-dependent selection. Space and time constraints necessitated blocking the experiment according to starting frequency. Densities of the two genotypes were estimated by destructively sampling ten microcosms five hours after inoculation, at which time experimental manipulations, if any, were performed (see below). The remaining forty microcosms were then sampled again after five further hours to obtain estimates of final densities.

We obtained fitness estimates for each treatment—competition alone, predation alone, the interaction between the two, and an antibiotic control—using the following procedure. 1 ml of a defined mixture of smooth and wrinkly-spreader genotypes obtained from pre-inoculation cultures grown overnight under shaken conditions in KB medium was inoculated into static microcosms containing 5 ml of KB at 28 °C. Experimental manipulations were performed five hours following inoculation, which is sufficient time for the initial formation of a biofilm. 1×10^4 cells per ml of *T. thermophila* (strain SB280) were added using a sterile syringe to puncture the biofilm without disturbing the spatial structure of the microcosm. To examine the effect of predation independently of competition, we also added 6.75 μg of the bacteriostatic antibiotic streptomycin just before adding the predator, as well as to control microcosms lacking the predator. By comparing the initial bacterial densities to the control we confirmed that this concentration of streptomycin prevents bacterial growth but does not kill the cells ($F_{1,81} = 0.46$, $P = 0.4998$). Additionally, the mean genotypic densities in the antibiotic treatment were used as the 'initial' densities when calculating r, to compensate for subtle non-significant effects of the antibiotics. Under these conditions, *T. thermophila* is resistant to streptomycin, and visual inspection using a microscope confirmed that protist behaviour remained unaltered. Visual observation of the microcosms also confirmed that the integrity of the biofilms was maintained during manipulation.

The large size of this experiment requires that we restrict attention to the interaction between a single pair of genotypes. Although genetic variation in frequency-dependent fitness among independently evolved wrinkly spreader genotypes has been observed[15,30], it is unlikely to represent a major source of bias here as our main concern was the relative, rather than absolute, effect of predation on frequency-dependent selection. Interestingly, our fitness estimate in the presence of predators predicted well the timing of diversification in an unrelated experiment (Fig. 3 and Supplementary Information), lending further support to the idea that these fitness estimates are unlikely to be severely biased.

Density of *T. thermophila* at the air–broth interface. Six replicate KB microcosms were inoculated with approximately 6×10^4 *T. thermophila* cells followed by the addition of 2 ml of mineral oil to the surface of three of the microcosms. 100 μl of medium was removed from the surface of each microcosm initially to ensure that each test tube was inoculated with the same concentration of *T. thermophila* ($t_4 = 0.0813$, $P = 0.9390$) and again after 4 h of static incubation at 28 °C. Counts were conducted using a haemocytometer.

Diversification in minimal media. Following ref. 15, we tracked the diversification of the ancestral smooth genotype (*P. fluorescens* SBW25) by destructively sampling static microcosms composed of M9 minimal medium supplemented with 3.14 g l^{-1} glucose with or without approximately 1×10^3 predators. Three replicate samples were removed every two days for 22 days. Prey diversity was estimated by plating on KB agar and noting the morphology of at least 100 colonies. *T. thermophila* densities were monitored by vortexing the microcosm for 45 s and counting a sample with a haemocytometer. Diversity was measured either as the complement of Simpson's index, $1 - \lambda = 1 - \sum p_i^2$ where p_i is the frequency of the ith type (following refs 13 and 16) or as richness, the number of morphologically distinct genotypes.

Wrinkly spreader fitness with and without predators. Five independently isolated and phenotypically distinct wrinkly spreader genotypes that had evolved in glucose microcosms with *T. thermophila* were competed separately against the ancestral smooth in minimal medium microcosms containing glucose as the sole source of carbon (as above) with and without predators. We inoculated 5 ml of media with approximately 1×10^4 c.f.u. ml^{-1} of a 1:1 by volume mixture of smooth and wrinkly spreader into microcosms containing 1×10^3 individuals of *T. thermophila* and control microcosms lacking the predator. The experiment was performed in triplicate and sampled after four days. At least 50 colonies of each type were counted to estimate genotypic densities, and the difference in the densities was used to compute the wrinkly spreader:smooth relative fitness (as above).

Rates of diversification. Diversity was estimated as for the minimal medium experiment outlined above but with KB medium and destructively sampling five replicate microcosms from each treatment every eight hours for five days, then once a day for another three days.

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