Experimental evolution of a microbial predator's ability to find prey

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Foraging theory seeks to explain how the distribution and abundance of prey influence the evolution of predatory behaviour, including the allocation of effort to searching for prey and handling them after they are found. While experiments have shown that many predators alter their behaviour phenotypically within individual lifetimes, few have examined the actual evolution of predatory behaviour in light of this theory. Here, we test the effects of prey density on the evolution of a predator's searching and handling behaviours using a bacterial predator, *Myxococcus xanthus*. Sixteen predator populations evolved for almost a year on agar surfaces containing patches of *Escherichia coli* prey at low or high density. Improvements in searching rate were significantly greater in those predators that evolved at low prey density. Handling performance also improved in some predator populations, but prey density did not significantly affect the magnitude of these gains. As the predators evolved greater foraging proficiency, their capacity diminished to produce fruiting bodies that enable them to survive prolonged periods of starvation. More generally, these results demonstrate that predators evolve behaviours that reflect at least some of the opportunities and limitations imposed by the distribution and abundance of their prey.

Keywords: experimental evolution; foraging theory; myxobacteria; predation

1. INTRODUCTION

Predators often have specialized traits that allow them to efficiently exploit their prey, from morphology that enables rapid pursuit of prey to venom used to incapacitate and digest prey. The usefulness of these traits is often obvious, but the selective forces that govern the optimization and quantitative contribution of these characters to fitness are difficult to study. How fast should a cheetah run to be maximally fit? How much venom should a viper deliver to despatch its prey in a time-efficient manner? The answers to such specific questions will, of course, depend on details unique to any particular predator–prey interaction. But more generally, certain aspects of the prey population—the density of prey, their diversity and relative quality, and their distribution—may similarly influence the evolution of predatory behaviours in many different systems. Foraging theory seeks to understand the relationship between properties of prey and a range of predatory behaviours (Schoener 1971; Stephens & Krebs 1986; Perry & Pianka 1997) including diet breadth (Emlen 1966; Sih & Christensen 2001; Catania & Remple 2005), when to forage socially (Giraldeau & Caraco 2000), how long to spend in a prey patch (Thiel & Hoffmeister 2004) and the effects of predation risk on these behaviours (Urban 2007). Such relationships may influence not only the evolution of predators in response to changes in their prey populations, but may also impact the structure of ecological communities (Thompson 1998; Bohannan & Lenski 2000; Yoshida *et al.* 2007).

Foraging theory typically assumes that predators behave in a manner that optimizes their rate of energy consumption in light of the availability of prey and other relevant constraints (Stephens & Krebs 1986; Perry & Pianka 1997). The theory has typically been tested by comparing model predictions with predator behaviours realized in experimentally manipulated environments. For example, in a classic study of the effect of prey density on diet breadth of the bluegill sunfish, Werner & Hall (1974) compared the proportion of different size classes of *Daphnia* that were consumed by these fish at high versus low overall prey densities. The foraging model they tested is based on a dichotomy in predatory behaviour between searching for prey and handling prey that have been captured. At low prey density, the model predicts that predators will consume even relatively low-profitability prey; whereas at high prey density, the model predicts that only the most valuable prey should be consumed. The experiments by Werner & Hall gave results that were qualitatively consistent with that theory. The behaviours exhibited by the fish presumably resulted from previous natural selection that shaped the plasticity of the fishes' behaviour in response to sensory information about the food availability in their immediate environment. However, in this study and in most other experiments that have tested foraging theory (Werner & Hall 1974; Charnov 1976; Krebs *et al.* 1978; Biesinger & Haefner 2005; Catania & Remple 2005), there was no evolution of the predator's behaviour over the experimental time scale.

There have been several empirical tests of theories mathematically related to foraging theory which have involved direct observation of evolution in bacteria–virus and bacteria–plasmid systems, and the results of these studies bear upon the evolutionary generality of foraging

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theory. For example, the density of susceptible hosts is expected to influence whether transmissible agents should evolve to become more benign, so that they can exploit individual hosts for longer periods of time, or whether they should evolve to exploit individual hosts more quickly, such that they can then infect additional hosts. The theory underlying these expectations is similar to the patch model of foraging theory. In some experiments of this type, viruses and plasmids that were deleterious to their hosts became more benign and even beneficial to their hosts when the transmissibility of the agents or the availability of susceptible hosts was limited during evolution (Bouma & Lenski 1988; Bull *et al.* 1991; Bull & Molineaux 1992; Lenski *et al.* 1994), an outcome consistent with theory. In another experiment, however, a plasmid's horizontal and vertical transmission rates did not evolve as expected when host abundance was manipulated (Turner *et al.* 1998). Recently, Heineman *et al.* (2008) and Guyader & Burch (2008) independently tested the predictions of foraging theory with respect to diet breadth by allowing populations of viruses to evolve on multiple strains of bacterial prey. Heineman *et al.* found that the viruses rapidly evolved the ability to discriminate between high and low quality prey, and the resulting restriction of diet breadth was host density dependent, as predicted by theory. Guyader & Burch (2008) found little evidence, however, for the effect of host densities on the evolution of the viruses in their experiments. Thus, some relationships between prey density and predator and pathogen behaviours have been tested by directly observing the evolution of viruses and plasmids. However, the predators in these previous experiments were not motile, and therefore the evolution of their searching behaviours *per se* could not be addressed. In this study, we also observe directly the evolution of a predatory micro-organism to test foraging theory, but we use a bacterial predator that is motile in order to examine the evolution of the searching versus handling components of its predatory behaviour.

A central component of foraging theory is the functional response, which describes the relationship between the density of prey and the predator's rate of consumption. The precise form of this response depends on many variables including the predator's behaviour, variation among prey organisms, and so on (Holling 1959; Tully *et al.* 2005). However, the typical 'type II' form of this response—in which the predator's consumption rate increases with a diminishing slope as its prey density increases—is critical to certain models that focus on the evolution of predatory behaviours, in particular the predator's allocation of time to searching for prey and handling them after they are found (Emlen 1966; Schoener 1971; Werner & Hall 1974; Charnov 1976; Krebs *et al.* 1978; Stephens & Krebs 1986; Perry & Pianka 1997; Sih & Christensen 2001; Biesinger & Haefner 2005; Catania & Remple 2005). At low prey densities, a predator spends most of its time searching for prey, and its consumption is more limited by its searching rate than by the speed with which it handles prey. Under such conditions, mutations that increase a predator's searching rate by, say, 10 per cent will be more beneficial than mutations that increase handling speed to the same extent. By contrast, at high prey densities, a predator spends relatively little time searching for prey; its consumption rate is limited more by its ability to handle prey, so that

mutations that improve handling speed are more beneficial than those that increase searching rate. Thus, foraging theory predicts greater improvement in the searching ability of predators that evolve in low-prey-density environments, whereas it predicts relatively greater evolutionary gains in their handling capability under high-prey-density conditions.

We tested these predictions in an experiment with evolving populations of *Myxococcus xanthus*, a predatory bacterium that preys upon a variety of other microbes, including bacteria and fungi. Swarms of *M. xanthus* cells search for prey by moving across surfaces (soil in nature, agar in the laboratory) using two genetically distinct motility systems (S- and A-motility) that differ in whether contact among cells is required for movement and in the mechanisms by which cells move (Hodgkin & Kaiser 1979a; Kaiser 2000; Mignot *et al.* 2007). Social (S) motility involves cell-cell interactions mediated by extracellular pili, whereas cells use adventurous (A) motility to move in isolation away from a swarm (Hodgkin & Kaiser 1979a,b). The relative contributions of these two motility systems to swarming depend on nutrient level and surface type (Shi & Zusman 1993; Hillesland & Velicer 2005), and they can also vary significantly among natural isolates (Vos & Velicer 2008). Cells at the swarm edge are the first to encounter new prey, and the relative contributions of A- and S-motility to swarm movement therefore influence whether prey are attacked by individual predators or groups.

Myxococcus xanthus secretes proteases, bacteriolytic enzymes and antibiotics that kill, digest and consume patches of prey (Rosenberg & Varon 1984) after they have been found, and investment in these activities must influence handling speed. *Myxococcus xanthus* may perform these activities even when prey are not around, but these extracellular functions would not kill prey unless they are performed in close proximity to the prey. Thus, searching and handling appear to be temporally exclusive activities, at least to a close approximation, as assumed by most models in foraging theory (Emlen 1966; Schoener 1971; Stephens & Krebs 1986; Perry & Pianka 1997; Sih & Christensen 2001). After the prey and resulting nutrients have been locally depleted, the *M. xanthus* predators use their motility systems and several inter-cellular signals to coordinate movement and construct fruiting bodies. Within these fruiting bodies, a portion of the population forms spores that can survive starvation, desiccation and other stresses (Dworkin 1996).

We evaluated whether searching and handling evolve differently depending on the density of prey by allowing several replicate populations of *M. xanthus* to evolve for almost 1 year on patches of a prey organism, *Escherichia coli*, which were arranged in either high- or low-density grids. During the experiment, *M. xanthus* populations were periodically transferred to fresh grids, where they moved by swarming while also reproducing vegetatively as they encountered prey patches. The overall fitness, searching ability and handling ability of the evolved populations were then compared directly with the same properties of their ancestors. As we will show, *M. xanthus* populations that evolved at low prey density exhibited significantly greater improvements in their search rate than those that evolved at high prey density, as predicted by foraging theory. Prey density had no discernible

effect, however, on the evolution of the predator's prey-handling ability.

2. MATERIAL AND METHODS

(a) *Evolution experiment*

Sixteen populations of *M. xanthus* were derived from two clones, GJV1 and GJV2, which differ only by a spontaneous rifampicin-resistance mutation (Velicer *et al.* 1998). The populations evolved in 12 cm square Petri plates (PGC Scientific) filled with 75 ml of TPM agar (1.5% agar plus TPM buffer (Bretscher & Kaiser 1978)). A grid of patches of the non-motile prey organism, *E. coli* B, was deposited on the agar using a 96-well plate and blotter (Hillesland *et al.* 2006). Prey patches were placed 1 and 2 cm apart to generate the high patch density (HPD) and low patch density (LPD) treatments, respectively. Four of the eight populations in each treatment used GJV1 as the founder, while four used GJV2. *Myxococcus xanthus* was added to a central prey patch and allowed to swarm outward for two weeks at 32°C. The incubator was humidified by a pan of water near the inflow fan; all plates in the evolution experiment and subsequent assays were kept in plastic bags, with slits cut in them, to minimize desiccation while allowing oxygen flow.

Predators were transferred to fresh plates every 14 days by scraping two perpendicular diameters of the swarm with a sterile dowel that was 1 mm in diameter. The end of the dowel was then rubbed in a central prey patch on the fresh plate. The prey used to inoculate each set of fresh plates came from a freezer stock; hence, the prey could not co-evolve with the predators. Predators experienced a total of 24 selection cycles. For two-thirds of the transfers, the perpendicular scrapings were centred on the patch that was initially inoculated, while for one-third they were offset by one patch.

This cross-sectional transfer method selected for genotypes capable of faster outward swarming, but probably less strongly than would have an outer-edge-only or even completely random sampling scheme. Vegetative myxobacteria cells reproduce as they move outward in search of new food, leaving their progenitors behind. Thus, the growth history of a swarm on an agar plate is distributed in concentric 'rings' of cells, with the oldest cells in the smallest rings in the centre and the newest cells in the largest, outermost rings. Thus, sub-populations of new mutants with increased fitness which arise as the swarm grows should be more frequent with increasing distance from the centre of the swarm. Our transfer regime would have sampled equal numbers of cells from all concentric rings because we scraped along the swarm diameters with a dowel of fixed width. Of course, the various ring sub-populations are not equal in size because each successive ring is larger than the preceding one. The newest, outermost ring, which should harbour the highest frequency of improved mutants, is much larger than the oldest, innermost ring, but they were sampled equally in absolute (not proportional) terms by the method that we employed. Improved mutants were under-sampled, therefore, relative to their frequency in the total swarm population, and such mutants were probably under-sampled to an even greater degree in the larger HPD swarms relative to the smaller LPD swarms. These considerations suggest that the evolution of improved swarming may have been impeded somewhat by the transfer protocol relative to one that employed completely random sampling, especially in the HPD treatment.

However, the HPD populations had larger population sizes and went through more generations than did the LPD populations (see below), even though these extra generations were the most under-sampled ones in the HPD lines. Thus, our transfer regime had the effect of diminishing (but not eliminating) the difference in effective number of generations between the HPD and LPD treatments. HPD lines still had greater opportunity for evolution than did the LPD lines, but the LPD lines nonetheless increased in searching ability to a greater degree than did the HPD lines, so these differences in opportunity cannot explain the treatment difference that we observed. Finally, these considerations do not take into account any differences in cell survival and viability across a swarm, although increased mortality towards the centre may have increased the representation of the outer rings among living cells at the time of transfer.

Owing to the strong tendency of *M. xanthus* to form clumps, it is difficult to obtain accurate cell counts needed to calculate the number of generations in our experiments; however, each transfer cycle represents at least 4 and perhaps as many as 12 cell generations (Hillesland 2005). Somewhat more generations elapsed under the HPD treatment than in the LPD treatment (owing to more prey to support reproduction in the former), so that the smaller fitness gains seen in the HPD treatment cannot be explained by fewer elapsed generations. Each evolving line was periodically sampled by scraping the entire population (less the cells transferred) off the plate, then depositing it on a slant containing CTT agar (1.5% agar and 1% casitone dissolved in TPM buffer; Bretscher & Kaiser 1978) plus 5 µg ml⁻¹ gentamicin to kill any surviving *E. coli*. After 3 days at 32°C, the sample was suspended in a solution containing three parts CTT plus gentamicin and one part 80 per cent glycerol, and the sample was frozen at -80°C. To initiate each assay of predatory traits, 50 µl of freezer stock of the ancestors and each evolved population were inoculated into CTT broth and allowed to acclimatize for 2 days at 32°C with constant shaking. Cultures were then transferred to fresh media, incubated another day, and each culture was centrifuged and re-suspended in TPM buffer to a density of 1 × 10⁹ cells ml⁻¹.

(b) *Assay of overall fitness*

Myxococcus xanthus reproduction in the evolution experiment requires that they encounter and consume prey; hence, the rate at which they encounter prey patches served as a proxy for overall fitness. To assess this rate, 10 µl of a predator suspension were added to a central patch of the relevant HPD or LPD plate. The number of patches encountered by the swarm was counted after two weeks, even if the swarm did not cover the entire patch. This number was then divided by the total number of patches (156 and 42 for HPD and LPD plates, respectively) to determine the encounter rate. This experiment included five temporal blocks; each block had four replicates of each ancestral clone and one replicate of each evolved population on both HPD and LPD plates.

(c) *Assays of searching and handling performance*

Searching and handling abilities were measured as the rates of swarm expansion on bare TPM agar plates and on confluent lawns of *E. coli* on TPM agar plates, respectively. These surfaces are the same as those that exist between and within prey patches, respectively. Ten µl of a predator suspension were added to the centre of a plate, and two perpendicular

Figure 1. Experimental design for evolution of a microbial predator at high and low densities of prey patches. The predator, *M. xanthus*, was added to one of the central patches of its prey, *E. coli*, in each environment. Photos were taken at (a) high and (b) low-prey-patch densities after 1 day. (c) To consume prey, a swarm of *M. xanthus* must search for and handle a prey patch; the swarm searches by moving across the surface between patches (dashed arrows), and the swarm handles (solid arrows) the prey by moving through a patch while killing and digesting the prey. After two weeks of incubation, swarms have spread outward and covered a greater proportion of patches at (d) high compared with (e) low patch density.

swarm diameters were measured after 3 and 14 days, with the resulting expansion rate calculated between those days. This experiment had two temporal blocks, each with eight replicates for each ancestral clone and two for each evolved population per surface.

(d) Statistical analyses

Statistical analyses were performed using the SAS software package (Cary, NC), v. 8.2. To calculate the relative values for overall fitness and the searching and handling components, each measurement of an evolved population was divided by a different randomly paired measurement of its ancestor from the same block. Each ratio was then log transformed, and we ran *t*-tests for each population to evaluate whether the mean log ratio was significantly different from zero, indicating evolutionary change. To test for effects across treatments, we ran mixed-model ANOVAs with the following model:

$$\text{Log ratio} = \text{evolenv} + \text{block} + \text{marker} \\ + \text{population}(\text{evolenv} \times \text{marker}),$$

where evolenv indicates the prey-density treatment effect; block denotes any influence of the temporal block; and marker is the effect of descent from GJV1 versus GJV2. Population refers to variation among the replicate lines within an evolutionary treatment and that effect is nested within the interaction between evolenv and marker. Block and population are random effects, while evolenv and marker are fixed effects.

3. RESULTS

(a) Evolution of predatory fitness

Myxococcus xanthus evolved on buffered-agar surfaces bearing patches of *E. coli* prey distributed at either high density (1-cm spacing between patches) or low density

(2-cm spacing between patches) for almost 1 year, with eight independent populations evolving in each density treatment (figure 1). Ancestral predators encountered many more prey in the HPD than in the LPD treatment over the two-week interval between transfers. Every two weeks, a dowel was used to sample cells from two perpendicular diameters of a predator 'swarm'—the outwardly moving and growing predator population—and the end of the dowel was rubbed into a central patch of prey on a fresh plate, which was then incubated for two weeks. Fresh prey populations for each cycle were prepared from a non-evolving frozen stock.

Escherichia coli was the only growth substrate for *M. xanthus* in both selective regimes. Thus, novel mutants able to reach and consume more prey patches than other genotypes could increase in frequency as the population on a plate swarmed outward, and hence would be represented at increasing frequencies in each successive subpopulation that was transferred. In both density treatments, natural selection should have favoured mutant predators that swarmed faster when cells were between patches, consumed prey more efficiently when cells were within patches or both. However, because predators in the low-density treatment spent more time and effort finding new prey patches than predators in the high-density treatment, selection should be relatively stronger on searching (between-patch swarming rate) in the low-density treatment than on handling (within-patch consumption rate), and vice versa for the high-density treatment.

After 24 two-week cycles, we tested whether the evolving predator populations had achieved higher overall fitness than their ancestor under the same prey-density treatment in which they had been selected. Technical difficulties, especially cell–cell adhesion, prevented direct

Table 1. Mean number and percentage of patches encountered by ancestor, LPD-evolved and HPD-evolved populations on low-density and high-density plates containing 42 and 156 total prey patches, respectively.

	no. of patches low density	percentage of patches low density	no. of patches high density	percentage of patches high density
ancestor	3.9	9.2	60	38
LPD-evolved	16	39	80	51
HPD-evolved	6.9	17	72	46

measurement of relative fitness by competing cultures of evolved and ancestral genotypes. As a proxy for overall fitness, we therefore compared the percentage of patches encountered in a given density treatment by pure cultures of evolved strains and their ancestor over a two-week period. This fitness proxy reflects the main component of overall adaptation in the evolution experiment, because the rate at which *M. xanthus* swarms encounter prey patches limits their rate of energy consumption and therefore their reproductive rate. We also compared the fitness of evolved lines relative to their ancestor in the alternative prey-density treatment in which they did not evolve. Table 1 shows the mean number and percentage of patches encountered by the ancestor and the evolved populations under each treatment.

Most evolved predators encountered more prey patches than did their ancestors in both prey-density environments, but the magnitude of improvement depended on both the environment in which the predator had evolved and the environment where its performance was measured. Figure 2 shows the overall fitness for each of the 16 evolved populations in both environments, in every case expressed relative to their ancestors in the same environment. All eight LPD-evolved predators improved significantly in the LPD environment (figure 2a), while only two of the HPD-evolved predators improved in that environment (figure 2b). Mean fitness gains were significantly greater for the LPD-evolved predators than for the HPD-evolved lines ($F_{1,13}=28.7$, $p=0.0001$). On average, the LPD-evolved predators found more than four times as many prey in the LPD environment as did their ancestors, whereas the HPD-evolved predators showed less than a twofold improvement in the LPD environment.

Most predators showed only slight fitness gains in the HPD environment (figure 2c,d; note the difference in scale relative to figure 2a,b). Seven of the eight populations in each evolutionary treatment showed a positive trend, but the fitness gains were small and they were significant in only four cases. Nonetheless, it is unlikely that so many populations would improve by chance (one-tailed sign tests, $p=0.0352$ for each treatment group alone and $p=0.0021$ for both groups combined), supporting the evolution of higher fitness even in the HPD environment. Under this regime, the mean fitness gain for the LPD-evolved populations was again higher than that for the HPD-evolved lines (approx. 1.4- and approx. 1.2-fold average gains, respectively), but this slight difference was not significant ($F_{1,13}=1.22$, $p=0.2888$).

(b) Evolution of searching and handling

Having shown that these predators evolved and improved, we now turn to experiments to address whether their fitness components of searching and handling changed in ways consistent with the expectations based on foraging theory. To encounter and consume a patch of prey, a swarm of *M. xanthus* must first move across the bare agar surface (at a searching rate), and then it must move through the prey patch while killing and digesting prey (at a handling rate), as shown in figure 1c. We have previously shown that when *M. xanthus* is placed in a prey patch, such that no searching is required, the rate of prey killing is very rapid (99% in a few hours) (Hillesland et al. 2006) relative to the time it takes the predators to move across the patch (1 day or more, data not shown). Thus, the rate at which a swarm of *M. xanthus* moves across bare agar provides a measure of the searching component of its overall fitness, while the rate at which it moves through a continuous lawn of prey measures the handling component of its fitness. *Myxococcus xanthus* populations clearly spent a greater proportion of their time searching for prey patches in the low-prey-density environment than they did in the high-prey-density environment. We expect, therefore, that selection for improved searching performance was more important in the low-prey-density treatment than in the high-prey-density regime. By extension, we also expect that selection for improved handling performance was more important in the high-prey-density treatment, at least on a relative basis compared with the other treatment. Table 2 summarizes data on the mean swarm radius and mean expansion rate of the ancestor and evolved populations in the searching and handling assays.

Overall, 15 of the 16 evolved populations had significantly faster searching rates than did their ancestors (figure 3a,b). The 7.2-fold average improvement by the LPD-evolved populations was significantly greater than the 2.4-fold average improvement by the HPD-evolved lines ($F_{1,13}=79.58$, $p<0.0001$). These data are therefore consistent with the expectation that a predator's searching performance should improve more when prey are scarce than when prey are common.

By contrast, the predicted effect of prey density on the evolution of handling performance was not well supported. Overall, there was a significant trend towards improved prey-handling speed (figure 3c,d), with 15 of the 16 evolved populations tending to be better than their ancestors (one-tailed binomial test, $p=0.0003$). However, the changes in this trait were small, and only one line showed significant improvement. Moreover, the prey-density treatment did not affect the evolution of the predator's handling ability ($F_{1,13}=0.94$, $p=0.3499$).

(c) Evolution of fruiting ability

More generally, evolutionary improvements in one aspect of an organism's performance may trade off with other fitness components (Levins 1968; Huey & Hertz 1984; Cooper & Lenski 2000; Bennett & Lenski 2007). In *M. xanthus*, certain genes have been shown to influence both predatory functions and fruiting-body formation (Pham et al. 2005). The formation of fruiting bodies that generate dormant, stress-resistant spores was probably not advantageous under the conditions of our evolution experiment, because additional prey patches were always

Figure 2. Evolutionary changes in the patch encounter rate of *M. xanthus* after almost 1 year at low or high prey density. The patch encounter rate serves as a proxy for overall fitness, as explained in the text. Each bar shows the log-transformed patch encounter rate of an evolved population relative to its ancestor. Error bars are 95% confidence intervals; asterisks denote significant fitness improvement ($p < 0.05$ after performing a sequential Bonferroni correction to adjust for eight tests in (a–d)). Rates for (a) LPD-evolved and (b) HPD-evolved predators measured in the low-prey-density environment. Rates for (c) LPD-evolved and (d) HPD-evolved predators measured in the high-prey-density environment; note the difference in the y-axis scale from the panels above.

Table 2. Mean swarm radii after 3 and 14 days, and the calculated expansion rate, on plates without (searching) and with prey (handling) for the ancestor, LPD-evolved and HPD-evolved populations.

	3-d search radius (mm)	14-d search radius (mm)	search rate (mm d ⁻¹)	3-d handle radius (mm)	14-d handle radius (mm)	handle rate (mm d ⁻¹)
ancestor	7.0	10	0.20	12	42	2.7
LPD-evolved	9.0	25	1.5	13	51	3.4
HPD-evolved	7.0	13	0.50	14	49	3.2

available to those swarms that continued to move outward and away from their fellow predators. We therefore compared fruiting-body development of the evolved populations with their ancestors to evaluate whether the gains in predatory performance were accompanied by declines in developmental performance. The ancestors produced numerous, large fruiting bodies within and around the central prey patch that was first inoculated (figure 4a,b). By contrast, all the evolved populations produced many fewer and much smaller fruiting bodies (figure 4c–f, and additional data not shown). This association is consistent with a trade-off between predatory and developmental fitness components in *M. xanthus*.

4. DISCUSSION

Foraging theory predicts greater improvements in searching rate for predators evolving at low prey densities, and greater improvements in handling performance when predators evolve at high prey densities. To test these predictions, we allowed 16 populations of the microbial predator *M. xanthus* to evolve on agar plates with patches of *E. coli* prey, either at low or high density, for 1 year. All of the evolved predators showed improvements in overall fitness and their rate of searching for prey patches, but

they all also suffered correlated losses in their ability to form fruiting bodies when subjected to starvation. The populations that evolved at low prey density exhibited faster searching rates than those that evolved at high density, as predicted by foraging theory. An evolutionary trend towards improved handling was also evident, but prey density did not affect the magnitude of those gains. While it is difficult to interpret this absence of a treatment effect, it is worth noting that overall fitness improvement was much greater in the *M. xanthus* populations that evolved in the low-prey-density treatment, where searching was more important, than in those populations that experienced the high-prey-density environment, where handling was more important (figure 2). Evidently, there was more scope for improvement in searching ability than in handling ability, given the properties of the ancestral strain and our experimentally imposed treatments.

More generally, this study represents a direct approach to testing the predictions of foraging theory. While many previous studies have examined behavioural responses of individual predators (for example, to changes in prey density), and while this plasticity was presumably shaped by natural selection, the experimental evolution of predator populations has only recently been employed as a means to test the predictions of foraging theory. In a

Figure 3. Evolutionary changes in searching and handling components of fitness. Searching performance of (a) LPD-evolved and (b) HPD-evolved predators; handling performance of (c) LPD-evolved and (d) HPD-evolved predators. Note the difference in scaling between (a,b) and (c,d). Searching and handling performances were based on swarming rates across surfaces without prey and with continuous prey lawns, respectively. Each bar shows the log-transformed ratio of an evolved population relative to its ancestor. Error bars and asterisks are defined as in figure 2.

conceptually related experiment also involving microbes (but not experimental evolution of new strains), *Abedon et al.* (2003) competed two viral strains, which differed in the speed with which they killed their bacterial hosts and the resulting number of viral progeny they produced, at different host densities. Their study showed that these differences in viral parameters influenced competitive fitness in a manner consistent with foraging theory; that is, the strain with the faster lysis, and lower yield per infected host, prevailed at high host densities. In a related study, *Heineman & Bull* (2007) used experimental evolution of bacteriophage T7 to test a model that predicts the optimal timing of lysis, which is conceptually related to the patch model in foraging theory. A population that was propagated at high host density evolved to initiate lysis earlier in infection, as predicted by the model, whereas lysis time did not shift towards the predicted optimum when phage evolved at low host density. *Heineman et al.* (2008) also used experimental evolution to generate viruses that could discriminate between high-quality and low-quality hosts. In a conceptually related experiment, however, *Guyader & Burch* (2008) found no compelling effect of host densities on the evolution of viral specificity.

In our study, we used a predatory bacterium to conduct a year-long evolution experiment to test directly the effect of prey density on the evolution of the predator's searching and handling behaviours. A predator's searching speed is predicted by foraging theory to have a greater impact on its

rate of energy acquisition at low prey density than at high density, where handling time represents a larger fraction of its temporal investment. Consistent with theory, searching rate improved significantly more in the predator populations that evolved at low prey density than in those that evolved at high prey density, while no significant effect was observed with respect to handling times. Nonetheless, we directly observed the evolution of different behavioural capacities in an active, motile predator as a function of environmental variation in the availability of its prey.

Our results also point to important future directions for research on *M. xanthus*. This experiment yielded 16 lines with a novel phenotype—enhanced swarming speed on bare agar—that has not (to our knowledge) been observed in mutant studies. Some evolved lines swarmed outwards at a rate almost 10 times faster than their progenitor, the widely studied *M. xanthus* strain DK1622. Given the relatively short period over which these evolutionary changes occurred (less than 300 generations), it seems likely that the same mutations that caused the searching-rate improvements also caused the losses in fruiting ability (*Cooper & Lenski* 2000). Indeed, there are known genetic connections in *M. xanthus* between fruiting-body development and predation (*Pham et al.* 2005). This developmental process requires cells to move towards regions of high population density when resources are scarce. In the patchy environment used in our evolution experiment, cells that did the opposite and moved away from their fellow predators would have encountered new prey

Figure 4. Fruiting-body development of ancestral and representative evolved predators. Photos were taken at $9\times$ magnification of the initially inoculated prey patch after two-week incubation for the following predators: (a) ancestor GJV1, (b) ancestor GJV2, (c) HPD-evolved line H2, (d) HPD-evolved line H4, (e) LPD-evolved line L1 and (f) LPD-evolved line L3. All photos were taken from low-prey-density plates, but differences between strains were qualitatively similar on high-prey-density plates.

patches before their competitors, and may thus have had an advantage. Future studies focusing on the genetic basis and physiological mechanisms that promoted faster searching will enable a fuller understanding of motility and the linkages between predation and development in *M. xanthus*.

Finally, experimental evolution with micro-organisms has already been used to test many important evolutionary issues, including the population-genetic mechanisms leading to the decay of unused traits (Cooper & Lenski 2000; Bennett & Lenski 2007), factors affecting the evolution of sex (de Visser *et al.* 1999; Goddard *et al.* 2005; Cooper 2007), and the relative importance of adaptation, chance and history in determining evolutionary outcomes (Travisano *et al.* 1995; Weinreich *et al.* 2006; Blount *et al.* 2008). *Myxococcus xanthus* has recently become an experimental model for the evolution of social behaviours (Velicer *et al.* 1998; Velicer *et al.* 2000; Velicer & Yu 2003; Fiegna *et al.* 2006), and our study demonstrates its potential as a model system for investigating the evolution of predator–prey interactions as well.

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