Coevolution alters predator life history traits, behavior and morphology in experimental microbial communities

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Abstract

Predator-prey interactions are key for the dynamics of many ecosystems. An increasing body of evidence suggests that rapid evolution and coevolution can alter these interactions, with important ecological implications by acting on traits determining fitness, including reproduction, anti-predatory defense and foraging efficiency. However, most studies to date have focused only on evolution in the prey species, and the predator traits in coevolving systems remain poorly understood. Here we investigated changes in predator traits after ~600 generations in a predator-prey (ciliate-bacteria) coevolutionary experiment. Predators independently evolved on seven different prey species, allowing generalization of the predator's evolutionary response. We used highly resolved automated image analysis to quantify changes in predator life history, morphology and behavior. Consistent with previous studies, we found that prey evolution impaired growth of the predator. In contrast, predator evolution did not cause a clear increase in fitness when feeding on ancestral prey. However, predator evolution affected morphology and behavior, increasing size, speed and directionality of movement, which have all been linked to higher prey search efficiency. These results show that in coevolving systems, predator adaptation can occur in traits relevant to offense level without translating into an increased ability of the predator to grow on the ancestral prey type.

Keywords: predator-prey interactions, coevolution, trait evolution, ciliate physiology, microbial model systems, experimental evolution

Introduction

Predator-prey interactions are ubiquitous and determine the dynamics of many ecosystems. Predation has been widely studied at an ecological level (Lotka 1925; Volterra 1926; Rosenzweig and Macarthur 1963), and recent research also shows that this interaction can be strongly altered by rapid evolution of anti-predatory defense in the prey (Yoshida et al. 2003) as well as by counter-adaptations in the predator (Brodie and Brodie 1999; Motychak et al. 1999; Hiltunen and Becks 2014), even though selection may be asymmetric resulting in slower evolutionary change for the predator (Dawkins and Krebs 1979). Moreover, owing to population growth-defense tradeoffs, rapid evolution of the prey and adaptation to predation can result in frequency-dependent selection of defended and undefended prey types as a function of predator population size (Meyer et al. 2006; Haafke et al. 2016; van Velzen and Gaedke 2017), an example of eco-evolutionary feedback dynamics. Common to this spectrum of evolutionary, coevolutionary and eco-evolutionary dynamics is that these dynamics are all driven by natural selection acting on fitness-relevant traits.

Predation can be described by three main phases, namely prey search, capture and ingestion (Matz and Kjelleberg 2005). These three phases are shaped by key traits in predator-prey systems, including those influencing offence and defense level, and all these traits can be subject to evolutionary change (Abrams 2000). Offense level is determined by sensory faculties and speed enabling location and capture of prey, and defense level by the capacity for predator avoidance and escape prior to ingestion as well as physicochemical obstruction of ingestion and digestion. Adaptations in defense and offense, in turn, combined with associated tradeoffs, modulate the reproduction (i.e. life history traits) of both parties. Examples abound of the study of the different phases of predation, and adaptation in both predator and prey life history traits. For example, the timing and population dynamics of many insectivorous bird species are tightly coupled to the dynamics of their prey insect species (Visser et al. 2012). Olive baboon sleeping site choice and behavior (sharing sleeping sites between multiple

baboon groups) in Kenya were recently linked to decreased contact and capture rate by leopards (Bidner et al. 2018). Coevolution has been hypothesized to occur between Northern Pacific rattlesnakes and California ground squirrels whereby venom resistance in squirrels is matched by increased venom effectiveness in rattlesnakes based on field data supportive of local adaptation of the traits (Holding et al. 2016). All of these empirical examples are, however, limited to a mostly comparative and behavioral ecology study approach, and cannot be used to experimentally investigate predator-prey coevolution due to the long generation times of the species.

Microbial systems offer a unique opportunity to study predator-prey dynamics, as they include efficient predators and allow for high replication as well as experimental approaches capturing both ecological and evolutionary dynamics. Microbial predator-prey systems show many key characteristics found also in other predator-prey systems, such as offense by speed (Visser 2007) and defense by avoidance of detection (Wildschutte et al. 2004), escape (Matz and Jurgens 2005), or physicochemical obstruction of ingestion or digestion (for an overview, see (Matz and Kjelleberg 2005)). Defense level has also been demonstrated to evolve in controlled setups (Lurling and Beekman 2006; Meyer and Kassen 2007). However, to our knowledge, there exist little to no empirical studies examining offense mechanisms subject to rapid evolution in microbial predator-prey systems.

Here we employed an experimental evolution approach to test the influence of ~600 generations of predator-prey coevolution on predator traits, using a microbial (ciliate-bacteria) model system. Since predator-prey dynamics are characterized by the intrinsically linked dynamics of both interaction partners, we inspected the influence of both prey and predator evolution on predator traits. To find general patterns in predator traits independently of any specific prey species, as most predators have multiple prey species, we used seven different prey species that were all separately coevolved with

the predator. Predator traits, analyzed using high-resolution video recording, aligned with theory on physiological factors increasing prey search efficiency for morphological and behavioral trait evolution (Crawford 1992; Visser 2007), although the patterns were often observed only for a subset of species. In contrast, life history traits showed both expected and unexpected patterns, such that prey evolution impaired predator growth as predicted while predator evolution failed to influence growth on the ancestral prey type (Gallet et al. 2009; Hiltunen and Becks 2014).

Material and methods

We studied coevolutionary dynamics of one focal predator species (the ciliate *Tetrahymena thermophila*) and seven of its bacterial prey species in all seven combinations of predator-prey species communities, as well as dynamics in prey-species populations only. Predator-prey dynamics and coevolution are relatively specific, but less constrained than host-pathogen dynamics, which often only contain a 1:1 species match while predators can frequently feed on multiple different prey species (Closs et al. 1999). We ran predator-prey coevolution experiments over about 600 predator generations, and assessed evolutionary effects on life history, morphology and behavior using common garden experiments.

Strains and culture conditions

The seven prey species used in this study are listed in Table 1. In addition to four taxa previously used as models in predator-prey studies, three strains were chosen based on representing genera associated with ciliate predators in natural habitats or potentially exhibiting different anti-predatory defense mechanisms (Table 1). Since all the strains represent unique genera, they are referred to by their genus name in the text. As a generalist predator, capable of consuming all the prey species, we used a single strain of the asexually reproducing ciliate *Tetrahymena thermophila* 1630/1U (CCAP) (Ketola et al. 2004).

Table 1. Bacterial strains used in this study.

Strain*	Rationale for species selection
Escherichia coli ATCC 11303	model prey (Hiltunen et al. 2017)
Janthinobacterium lividum HAMBI 1919	pre-/post-ingestion defense: toxin release (Matz and Kjelleberg 2005)
Sphingomonas capsulata HAMBI 103	model prey (Hiltunen and Laakso 2013)
Brevundimonas diminuta HAMBI 18	realistic habitat (Becks et al. 2005)
Pseudomonas fluorescens SBW25 (Bailey et al. 1995)	model prey (Hiltunen et al. 2018)
Comamonas testosteroni HAMBI 403	pre-ingestion defense: oversize (Matz and Kjelleberg 2005)
Serratia marcescens ATCC 13880	model prey (Hiltunen and Laakso 2013)

*ATCC = American Type Culture Collection; HAMBI = HAMBI mBRC = Microbial Domain Biological Resource Centre HAMBI, University of Helsinki, Finland

Prior to the experiments, all bacterial stocks were kept at –80 °C and ciliate stocks were cultured axenically in proteose peptone yeast extract (PPY) medium containing 20 g of proteose peptone and 2.5 g of yeast extract in 1 L of deionized water. During the coevolutionary experiment, cultures were kept at 28 °C (± 0.1 °C) with shaking at 50 r.p.m.

Predator-prey coevolutionary experiment

The coevolution experiment was started using a small aliquot (20 μL) of a 48-h bacterial culture started from a single colony and 10,000 ciliate cells (approx. 1,700 cells mL^{-1}) from an axenic culture. Each bacterial strain was cultured alone and together with the ciliate predator (three replicates each, with the exception of six replicates for *Comamonas*) in batch cultures of 20 mL glass vials containing 6 mL of 5 % KB medium, with 1 % weekly transfer to fresh medium.

Every four transfers (28 days), bacterial and predator densities were estimated using optical density (1 mL sample at 600 nm wavelength) as a proxy for bacterial biomass and direct ciliate counts (5 × 0.5 μL droplets using light microscopy) as described previously (Cairns et al. 2016), and samples were freeze-stored with glycerol at –20 °C for later analysis. Since predators do not survive freeze-storage in these conditions, at time points 52 and 89 weeks, predator cultures were made axenic by transferring 400 μL into 100 mL of PPY medium containing an antibiotic cocktail (42, 50, 50 and 33

μg mL^{-1} of kanamycin, rifampicin, streptomycin and tetracycline, respectively) and stored in liquid nitrogen. Axenicity was controlled for by plating on agar plates containing 50 % PPY medium where all experimental bacterial strains grow. The liquid nitrogen storage protocol was modified from a previously used protocol (Cassidy-Hanley 2012) and included starving a dense ciliate culture in 10 mM Tris-Hcl solution (pH 7) for 2–3 days, centrifugation (1700 g, 8 min, 4 ºC), resuspension of the pellet in 1 mL of leftover supernatant, and the addition of 4 mL of sterile 10 % DMSO. The resultant solution was transferred to cryotubes in 0.3 mL lots, and frozen in a –20 °C freezer at a rate of –1 °C/minute using a Mr. Frosty™ Freezing Container (Thermo Scientific) for cell preservation before transferring to liquid nitrogen.

Sample collection and preparation

We isolated the populations for the current experiment at time point 89 weeks (approx. 20 months). With the minimal assumption that populations multiply by 100-fold (dilution rate) until reaching the stationary phase, each weekly transfer interval represents 6.64 generations for both prey and predator (Lenski et al. 1991), constituting a total minimum of ~600 generations. Community dynamics are shown in Supplementary Figures S1 and S2 and show clear differences in population size between different prey species.

Bacteria were restored from freeze-storage by transferring 20 μL into 5 mL of 5 % KB medium and culturing for 72 h. Predators were restored from liquid nitrogen by thawing cryotubes in a 42 °C water bath for 15 s, followed by the addition of 1 mL of 42 °C PPY medium. The cryotube contents were then transferred a petri dish containing PPY medium at room temperature. Upon reaching high density (approx. 48 h), predators were transferred to 100 mL of PPY medium and cultured to high density (approx. seven days). To ensure that the antibiotic treatment or the liquid nitrogen storage and revival procedures do not contribute to potential differences between the ancestral predator and

evolved predator lines, the axenic ancestral predator was subjected to identical procedures and was revived at the same time as the evolved lines. These culturing steps representing over 10 generations should remove the influence of non-genetic changes in predator traits caused by phenotypic plasticity (Fronhofer and Altermatt 2015).

Physiological measurements

To test bacterial and ciliate performance and traits, we used a combination of automated video analysis, optical density measurements and flow cytometry. To separate evolutionary responses on the predator and prey level, we tested performance of both evolved and ancestral bacteria with evolved and ancestral ciliates for all coevolved lines reciprocally. To do so, we prepared 12 50 mL falcon® tubes by adding 20 mL of 5 % KB medium. Three of these were inoculated with ancestral bacteria and ancestral ciliates, three with ancestral bacteria and evolved ciliates, three with evolved bacteria and ancestral ciliates and the remaining three with evolved bacteria and evolved ciliates. We placed the falcon® tubes in a 28 °C incubator, rotating on a shaker at 120 r.p.m. After inoculation, the samples were left to grow for a period of 12 days, to allow populations to grow to equilibrium density. Over the course of these 12 days, we took a total of 10 samples from each culture for analyzing population density dynamics of bacteria and ciliates, and morphological and behavioral metrics for the ciliates.

Bacterial density measurements

Bacterial density was determined through measurement of both optical density and through flow cytometry following established protocols (Hammes et al. 2008). For flow cytometry, we sampled 50 μL of all cultures, diluted 1:1000 using filtered Evian water and transferred 180 μL of the diluted samples to a 96-well-plate. We then added 20 μL of SybrGreen to strain the cells and measured bacterial cell counts using a BD Accuri™ C6 flow cytometer. The full protocol can be found in

Supporting Information. For optical density measurement, we sampled 50 μL of all cultures, diluted 1:10 using filtered Evian water, and measured absorbance at 600 nm using a SpectroMax 190 plate reader.

Ciliate density and trait measurements

For measuring ciliate density, we used a previously established method of video analysis (Altermatt et al. 2015) using the BEMOVI R-package (Pennekamp et al. 2015). We here followed a previously established method (Fronhofer et al. 2017b) where we took a 20 s video (25 fps, 500 frames) of a standardized volume using a Leica M165FC microscope with mounted Hamamatsu Orca Flash 4.0 camera. We then analyzed the videos using BEMOVI (Pennekamp and Schtickzelle 2013; Altermatt et al. 2015), which returns information on the cell density, morphological traits (longest and shortest cell axis length) and movement metrics (gross speed and net speed of cells, as well as turning angle distribution). The video analysis script, including used parameter values, can be found in the Supporting Information.

Data analysis

All statistical analyses were done using the R statistical software (version 3.5.1).

Predator trait space

To visualize whether the full set of trait data displayed structure depending on the evolutionary history of the predator and prey species, t-distributed stochastic neighbor embedding (t-SNE) was performed for each prey species separately using the Rtsne package (van der Maaten 2014) with a perplexity parameter of 3.

Beverton-Holt model fit

For analyzing the population growth dynamics of the ciliates, we implemented the Beverton-Holt population growth model (Beverton 1957) (Figure S3) using a Bayesian framework in Rstan, following methods used by (Fronhofer 2018; Rosenbaum et al. 2019). This function has the form of:

$$\frac{dN}{dt} = \left(\frac{r_0 + d}{1 + \alpha N} - d\right) N$$

With r_0 being the intrinsic rate of increase, α the intraspecific competitive ability and d being the death rate in the population. Model code for fitting this function can be found on a Github repository (doi: 10.5281/zenodo.2658131). For fitting this model, we needed to provide prior information for r_0, d and equilibrium density K. The intraspecific competitive ability α was later derived from the other parameter values as:

$$\alpha = \frac{r_0}{K * d}$$

The priors (lognormal distribution) of the model were chosen in such a way that mean estimates lay close to the overall observed means, but were broad enough so the model was not constrained too strongly.

- Equilibrium population density K: $ln(K) \sim normal(9.21, 0.5)$
- Intrinsic rate of increase r0: $ln(r0) \sim normal(-2.3, 0.5)$
- Rate of mortality d: $ln(d) \sim normal(-2.3, 0.5)$

Models were run with a warmup of 2,000 iterations and a chain length of 8,000 iterations.

Life history trait analysis

We analyzed the estimates of the life history traits obtained from the Beverton-Holt model fit (r_0, α, and K) using linear models and model selection. We first constructed a full model with life history traits being a function of bacterial evolutionary history (evolved/ancestor), ciliate evolutionary history (evolved/ancestor) and bacterial species (seven species factors) in a full interaction model. Next, we used automated bidirectional model selection using the step function (stats package version 3.5.1) to find the best model. To avoid bias due to starting point, we fit the model both starting from the intercept model and the full model, and if model selection resulted in different models, we used AICc comparison (MuMin R-package, version 1.42.1) to select the model with the smallest AICc value.

Morphological and behavioral trait analysis

Morphological and behavioral data was available for every time point during the growth curve, and since we know these traits can be plastically strongly affected by density (Fronhofer et al. 2015; Fronhofer et al. 2017a), we had to take density into account in the model. We hence separated the analysis into two steps: first, we identified key points in the growth curves (early phase, mid-log phase and equilibrium density phase) and analysed the traits for these particular points. Secondly, we fit models over all data, but taking bacterial and ciliate densities into account as covariates in the statistical analysis.

We defined the early phase as the second time point in the time series, equilibrium density phase as the first time point where density was larger than 99 % of K, or alternatively the highest density, and the mid-log phase as the point between the early and equilibrium density phase where density was closest to 50 % of K. We then created statistical models for the traits (major cell axis size, gross speed of cells and turning angle distribution) as a function of bacterial evolutionary history (evolved/ancestor), ciliate evolutionary history (evolved/ancestor) and bacterial species (seven

species factors) including a full interaction for the data at the particular time point. Next, we used automated bidirectional model selection to find the best fitting model. This was done separately for all three phases (early, mid-log and equilibrium density phase). We again performed model selection both starting from the intercept model and full model, and compared the 2 models using AICc comparison to identify the best model.

We then created models using all the data, where we fit major cell axis size, gross speed and turning angle distribution as a function of bacterial evolutionary history (evolved/ancestor), ciliate evolutionary history (evolved/ancestor) and bacterial species (seven species factors), ciliate population density (ln-transformed, continuous) and bacterial population density (ln-transformed, continuous), including a full interaction. For turning angle, we also did a log10 transformation of the turning angle distributions, as fitting the model on untransformed data leads to a strong deviation on the qqplot. Next, we used automated bidirectional model selection using the step function starting from intercept model and full model, and compared the 2 models using AICc comparison to select the best model.

Results

The t-SNE maps (Figure 1) showed that the evolutionary history of the predator and prey species frequently resulted in predator divergence in trait space. Importantly, this divergence evolved from a single ancestral predator population, which was subjected to coevolution with different prey species. There were also prey species specific patterns in the relative contribution of predator evolutionary history versus prey evolutionary history to trait space divergence.

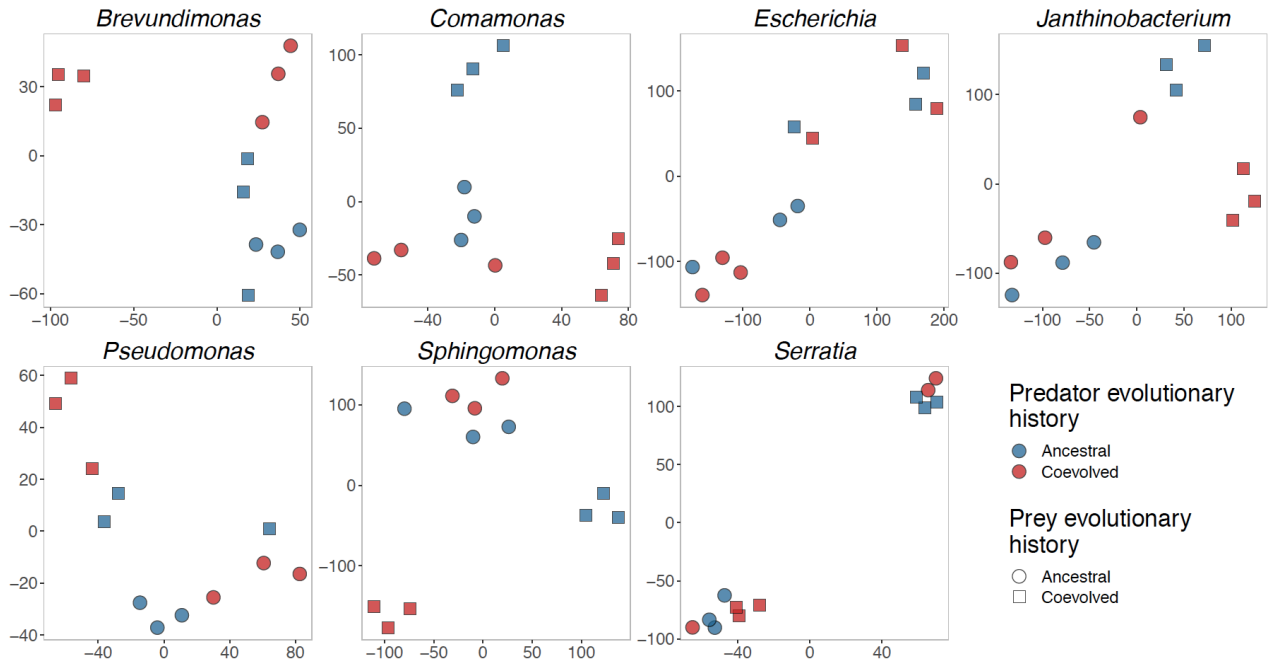

Figure 1. t-SNE map of contribution of predator and prey evolutionary history to predator divergence in trait space. The traits included in the analysis encompass life history (intrinsic growth rate, equilibrium density and competitive ability), morphology (cell size and biovolume) and behavior (speed and cell turning angle distribution).

Prey evolution drove changes in the life history traits of the predator, including intrinsic rate of increase (r_0), equilibrium density (K) and competitive ability (α), although the presence and strength of the effect depended on the bacterial species (ANOVA for LM on r_0: prey evolution $F_{1,78} = 29.8$, $p < 0.001$; prey evolution × prey species $F_{6,78} = 9.03$, $p < 0.001$; K: prey evolution $F_{1,80} = 0.33$, $p = 0.57$, prey evolution × prey species $F_{6,80} = 13.7$, $p < 0.001$; α: prey evolution $F_{1,78} = 15.1$, $p < 0.001$, prey evolution × prey species $F_{6,78} = 5.48$, $p < 0.001$; for full results, see Supplementary Tables S1–S3; Figure 2). The differences observed always showed decreased growth properties in the predator when feeding on evolved prey lines which displayed, on average, 0.76, 0.98 and 0.77 times the growth rate, equilibrium density and competitive ability, respectively, of the ancestral lines. Specific differences include decreased ciliate growth rate for evolved *Comamonas* and *Sphingomonas*, decreased ciliate

equilibrium density for evolved *Escherichia*, *Pseudomonas* and *Sphingomonas*, and decreased ciliate competitive ability for evolved *Comamonas*.

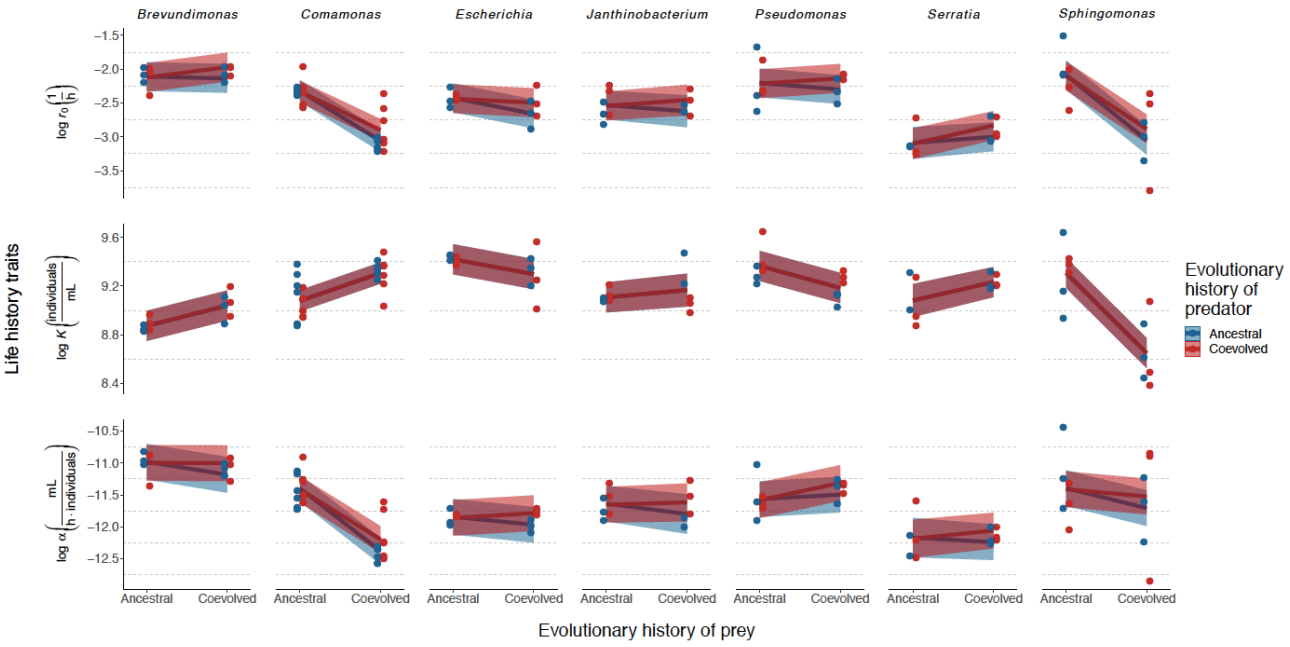

Figure 2. Reaction norms showing effect of predator-prey coevolution on life-history traits of predator (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). The life-history traits for predators are parameters of Beverton-Holt continuous-time population models fitted to data, and include intrinsic growth rate (r_0), equilibrium density (K) and competitive ability (α). The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

In contrast to life history traits, which were affected by prey evolution alone, morphological and behavioral traits of the predator were affected by predator evolution (Figure 3). Evolved predators were slightly but significantly (on average, 1.5-fold) larger than unevolved predators (ANOVA for LM on cell size: predator evolution $F_{1,767} = 6.59$, $p = 0.010$), although there was a marginally significant effect indicating that this was modulated by the evolutionary history of the prey species (ANOVA for LM on cell size: prey evolution $F_{1,767} = 2.97$, $p = 0.085$). The effect also depended on

predator and prey densities such that the strongest differences in cell size between ancestral and coevolved predators were observed at low prey densities (ANOVA for LM on cell size: prey evolution $F_{1,767} = 28.1$, $p < 0.001$; full results, see Supplementary Table S4 and Figures S4–S6).

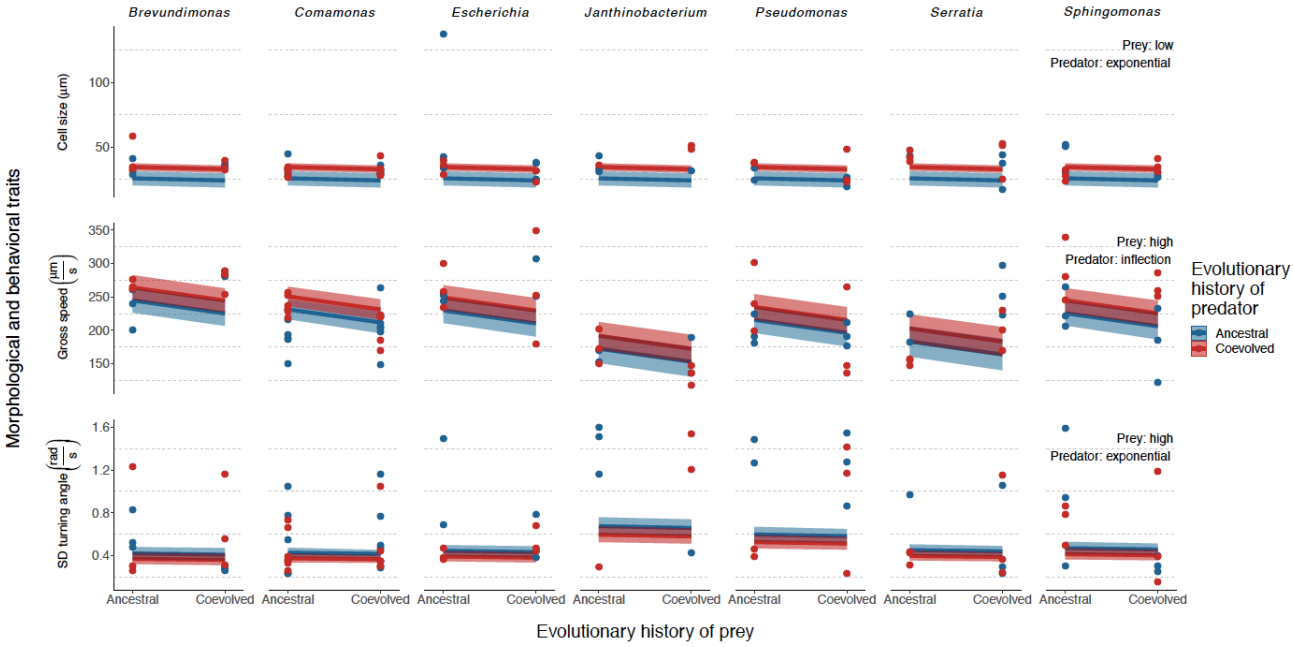

Figure 3. Reaction norms showing effect of predator-prey coevolution on morphology and behavior of predator (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Bacterial and ciliate densities have been chosen to show conditions where predator evolution changes traits, and are indicated for each trait in the upper right-hand corner. Low and high prey density represent 5 % and 95 % quantiles, respectively. "Inflection" represents predator density at the inflection point and "exponential" predator density at the exponential phase, estimated using Beverton-Holt population models. For details concerning whether and how predator density, prey density and prey species influence the effects, see the main text and Supplementary Information, which includes figures for all relevant predator and prey densities for each trait. Cell turning angle distribution (standard deviation, SD) is used as a proxy for directionality of cell movement which is higher at lower values. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

The gross movement speed of predators depended on the interplay between predator density and predator or prey evolutionary history, although evolved predators had, on average, 1.2-fold higher

speed compared to ancestral predators. Specifically, evolved predators maintained higher speed at increasing predator densities (ANOVA for LM on gross speed: predator density $F_{1,763} = 94.9$, $p < 0.001$; predator evolution $F_{1,763} = 3.13$, $p = 0.077$; predator evolution × predator density $F_{1,763} = 3.98$, $p = 0.046$) which was counteracted by prey evolution by driving speed to a lower rate at increasing predator densities (ANOVA for LM on gross speed: prey evolution $F_{1,763} = 2.94$, $p = 0.086$; prey evolution × predator density $F_{1,763} = 5.46$, $p = 0.020$; for full results, see Supplementary Table S5 and Figure S7). Finally, predator evolution altered cell turning angle distribution, which is a proxy for the directionality of cell movement (ANOVA for LM on cell turning angle distribution: predator evolution $F_{1,763} = 7.33$, $p = 0.007$), across prey species such that evolved predator lines moved in straighter trajectories (on average, 0.94 times the turning angle distribution) compared to the ancestral predator in the exponential growth phase (ANOVA for LM on cell turning angle distribution: predator density $F_{1,763} = 152$, $p < 0.001$; for full results, see Supplementary Table S6 and Figures S8–S10),

Discussion

We quantified the contribution of predator and prey evolution to predator trait change across seven different prey species in a 20-month (~600 predator generations) coevolutionary experiment. We expected rapid evolution of anti-predatory defense in the prey to cause impairment of predator growth (Hiltunen and Becks 2014; Huang et al. 2017). We expected predator evolution to be weaker in line with the life-dinner principle positing that the prey experiences stronger selection pressure since its survival (life) directly depends on defense while the predator can afford a certain measure of unsuccessful prey encounters (dinner postponement) (Dawkins and Krebs 1979; Vermeij 1994). Asymmetric selection can result in dynamics other than classic arms race dynamics such as frequency-dependent cycling of traits (Brodie and Brodie 1999), which have also been observed in microbial predator-prey systems (Meyer and Kassen 2007). Nevertheless, instead of escalation where predators alone impose selection pressure, we expected to also observe predator evolution, since

coevolution has been demonstrated to occur in bacteria-ciliate systems (Gallet et al. 2009; Hiltunen and Becks 2014; Huang et al. 2017).

Prey evolution led to hypothesized changes in predator life history traits, decreasing intrinsic growth rate, equilibrium density and competitive ability, while not affecting morphological or behavioral traits in the predator. Interestingly, the strength of the effect and the life history trait affected depended on the prey species. These results may be influenced by different growth dynamics (Figures S1 and S2), defense levels or defense mechanisms (Matz and Kjelleberg 2005) of the different prey species. Remarkably, against our expectation, we did not find detectable levels of adaptation in predator life history traits when prey-evolved predators fed on their respective ancestral prey species. This could be indicative of asymmetry of selection (Brodie and Brodie 1999; Meyer and Kassen 2007) such that predators experience weaker selection pressure compared to prey. Slow evolutionary change for ciliate predators could also result from smaller population size (in the order of 10^4 mL^{-1} for ciliates compared with 10^8 mL^{-1} for bacteria), larger genome size (>100 Mb for *T. thermophila* (Eisen et al. 2006) compared to <10 Mb for bacteria) or more complex genomic architecture limiting adaptive mutation supply compared to the bacterial prey. Alternatively, since improved predator growth on ancestral prey has been observed in shorter-term experiments (Gallet et al. 2009; Hiltunen and Becks 2014), growth-offense tradeoffs exacerbating over time may impose constraints on life history trait adaptation in coevolving systems in the long term (Huang et al. 2017). It is also possible that counter-adaptations to evolved defense mechanisms, such as cell aggregation (Lurling and Beekman 2006; Meyer and Kassen 2007), fail to confer an advantage to the predator when feeding on the ancestral prey.

Despite a lack of adaptation in life-history traits, coevolved predators displayed both behavioral and morphological changes. Namely, increased swimming speed and body size were observed for

coevolved predators with certain prey species. Increased swimming speed may increase prey search efficiency (Crawford 1992; Visser 2007). The role of increased body size is less clear but has been linked to the same trait since swimming speed can be a function of body size (Crawford 1992; Visser 2007). Notably, predators evolved to swim in straighter trajectories across prey species, also hypothesized to increase prey search efficiency (Zollner and Lima 1999; Visser 2007).

Our findings have implications for interpreting data from coevolving predator-prey systems. First, the pronounced impairment of predator growth traits upon prey evolution together with the lack of clear improvements in the ability of coevolved predators to feed on ancestral prey types corroborate the asymmetric selection hypothesis. Second, the occurrence of predator evolution in other key traits for predator-prey interaction despite this suggests that tracking ecological changes alone may result in an underestimation of predator (co)evolution. Further studies are required to identify the factors producing this constraint, such as offense-growth tradeoffs or the specificity of the advantage of improved offense to defended prey types.

Data Availability

All code and pre-processed data needed to reproduce the ecological and evolutionary analyses will be available via Dryad.

Author Contributions

Designed coevolutionary experiment: J.C. and T.H. Performed and managed experiment: J.C. Designed physiological measurements: F.M., E.A.F., and F.A. Performed physiological measurements: F.M., E.A.F., and F.A. Analyzed data: F.M. and J.C. Wrote manuscript draft: J.C. All authors interpreted results and participated in improving the manuscript.

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Conflict of Interest Statement

We declare we have no competing interests.

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