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Host mating system and coevolutionary dynamics shape the evolution of parasite avoidance in *Caenorhabditis elegans* host populations

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Research Article

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Abstract

Hosts exhibit a variety of defence mechanisms against parasites, including avoidance. Both host–parasite coevolutionary dynamics and the host mating system can alter the evolutionary trajectories of populations. Does the nature of host–parasite interactions and the host mating system affect the mechanisms that evolve to confer host defence? In a previous experimental evolution study, mixed mating and obligately outcrossing *Caenorhabditis elegans* host populations adapted to either coevolving or static *Serratia marcescens* parasite populations. Here, we assessed parasite avoidance as a mechanism underlying host adaptation. We measured host feeding preference for the coevolved and static parasites *vs* preference for *Escherichia coli*, to assess the evolution of avoidance behaviour within our experiment. We found that mixed mating host populations evolved a preference for *E. coli* relative to the static parasite strain; therefore, the hosts evolved parasite avoidance as a defence. However, mixed mating hosts did not exhibit *E. coli* preference when exposed to coevolved parasites, so avoidance cannot account for host adaptation to coevolving parasites. Further, the obligately outcrossing host populations did not exhibit parasite avoidance in the presence of either static or coevolved parasites. Therefore, both the nature of host–parasite interactions and the host mating system shaped the evolution of host defence.

Introduction

Parasites are capable of imposing strong selection on host populations, often resulting in the evolution of host defence mechanisms (Thompson, 1994, 2005). Host populations can exhibit a diverse array of defences that confer increased fitness in the presence of parasites. Multiple molecular pathways enable cellular immune responses in both the adaptive (Litman *et al.* 2010) and innate (Engelmann and Pujol, 2010; Buchon *et al.* 2014) immune systems of hosts. Further, hosts may also engage in behavioural defences against parasites. Behavioural defence includes medicinal behaviours that clear or impede existing infections (Chapuisat *et al.* 2007; Singer *et al.* 2009; Lefevre *et al.* 2010) and parasite avoidance, wherein the host engages in actions that limit its exposure to the parasite (Taylor, 1954; Dudley and Mitton, 1990; Feener and Moss, 1990; Hart, 1990, 1994; Chang *et al.* 2011; Moore, 2012; Meisel and Kim, 2014). Given this diversity of host responses, many factors may shape the evolution of host defence. Do specific host traits consistently evolve to confer defence for a given host–parasite interaction, or is the evolution of host defence influenced by the context of the interaction?

Both the host mating system and host–parasite coevolutionary dynamics can significantly alter the evolutionary trajectories of host populations, which may then shape the evolution of host defence. With regard to mating systems, host outcrossing can facilitate rapid adaptation to parasites, whereas self-fertilization can impede adaptive evolution (Busch *et al.* 2004; Morran *et al.* 2009, 2011, 2013; Teotonio *et al.* 2012; Masri *et al.* 2013; Carvalho *et al.* 2014; Slowinski *et al.* 2016). Selection imposed by coevolving parasites may favour host outcrossing over self-fertilization for several reasons that are not mutually exclusive. First, if coevolving parasites impose negative frequency-dependent selection, then outcrossing may generate host progeny with rare genotypes (Jaenike, 1978; Hamilton, 1980; Bell, 1982). This is the basis for the Red Queen hypothesis (reviewed in Lively and Morran, 2014). Second, outcrossing may increase the efficacy of selection on certain loci, relative to self-fertilization, by breaking linkage disequilibrium (Fisher, 1930; Muller, 1932; Hill and Robertson, 1966; Felsenstein, 1974). Under this scenario, outcrossing should facilitate greater rates of adaptation than self-fertilisation by uniting multiple beneficial mutations into a common host genome more rapidly (reviewed in Hartfield and Keightley, 2012). Further, by increasing the efficacy of selection across the genome, outcrossing may increase the likelihood of evolving multiple host defences in response to selection acting simultaneously on different traits. Therefore, the host mating system can significantly alter the evolution of the host genome in response to selection and consequently host defence may evolve differently under different mating systems. Specifically, outcrossing populations may have a greater capacity to evolve significantly enhanced host defence, potentially via multiple mechanisms, relative to predominantly selfing populations.

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Avoidance can be a very effective first line of defence against parasites (Hart, 1990, 1994). However, avoidance requires hosts to detect the parasite; therefore, some level of recognition, and perhaps specificity, between host and parasite populations should be critical for hosts to exhibit avoidance as a defence (Meisel and Kim, 2014). Local adaptation occurs when host, parasite or host and parasite populations evolve specificity towards their sympatric antagonist population, as opposed to the evolution of a response to individuals of the antagonist species in general. Antagonistic coevolutionary interactions, as opposed to transient or static interactions, can promote specificity between hosts and parasites (Ebert, 1994; Kaltz and Shykoff, 1998; Lively and Dybdahl, 2000; Laine, 2005, 2007; Morgan *et al.* 2005; Greischar and Koskella, 2007; Gibson *et al.* 2015). Therefore, antagonistic coevolution may favour more rapid evolution of avoidance behaviour than host interactions with transient or static parasite populations. Additionally, the evolution of specificity in host–parasite interactions can be accelerated by host outcrossing (Morran *et al.* 2014). Therefore, outcrossing host populations may be more likely to evolve avoidance as a response to selection imposed by coevolving parasites than selfing hosts.

In a previous study, obligately outcrossing and mixed mating host populations of the nematode *Caenorhabditis elegans* were exposed to either coevolving or static (non-coevolving) populations of the bacterial parasite *Serratia marcescens* in the presence of their normal laboratory food source, *Escherichia coli* (Morran *et al.* 2011). *Serratia marcescens* can rapidly kill *C. elegans* upon consumption (Schulenburg and Ewbank, 2004). However, paradoxically, *C. elegans* hosts generally prefer to feed on *S. marcescens* rather than some non-parasitic strains of *E. coli* (Zhang *et al.* 2005; Pradel *et al.* 2007; Glater *et al.* 2014). Hosts were plated directly onto a lawn of *S. marcescens* and were required to crawl to *E. coli* for survival and reproduction. After 30 generations, the host populations exhibited elevated levels of fitness in the presence of the parasites, and the obligately outcrossing populations exhibited greater rates of adaptation than the mixed mating populations (Morran *et al.* 2014; Penley *et al.* unpublished result). Further, the parasites that coevolved with the obligately outcrossing populations exhibited greater host specificity than the parasites that coevolved with mixed mating hosts (Morran *et al.* 2014). We hypothesized that selection to avoid the parasite may have been strong during experimental evolution, and that host defence may have evolved differently depending on the host mating system and the nature of host interactions with the parasite. Here, our goal was to determine if adaptation in the host populations was driven by the evolution of parasite avoidance. Using our experimentally evolved populations, we measured *C. elegans* host preference for *E. coli vs S. marcescens* parasite populations to test for the evolution of parasite avoidance within the context of our previous experiment. Additionally, we assessed whether the host mating system and host–parasite coevolutionary dynamics influenced the evolution of parasite avoidance as a host defence.

Methods and materials

Host populations

Host populations consisted of five mixed mating and five obligately outcrossing *C. elegans* populations derived from strain PX382 with an overall CB4856 background, obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, Minnesota, USA). Obligately outcrossing populations were modified with the *fog-2*(*q71*) mutation, which prevents hermaphrodites from producing sperm, requiring outcrossing for reproduction (Schedl and Kimble, 1988). Each of the five replicate populations within each mating type were independently exposed to ethyl methanesulphonate (EMS) mutagenesis to infuse novel genetic variation prior the start of the experiment, and then each population was split and replicated across each treatment (Morran *et al.* 2011). A subset of each population was frozen at −80 °C prior to experimentation and preserved prior to experimental evolution, the ancestral population for a given experimental population.

Parasite populations

Serratia marcescens (Sm2170) is a gram-negative bacterium and virulent parasite of *C. elegans* that causes systemic infection and ~80% mortality within 24 h upon consumption (Schulenburg and Ewbank, 2004). Sm2170 can impose strong selective pressure against *C. elegans* (Morran *et al.* 2009). *Serratia marcescens* can be recovered from the *C. elegans* gut and cultured, facilitating experimental coevolution (Morran *et al.* 2011).

Experimental evolution

Each host population was split across three treatments for experimental evolution: evolution, coevolution and control for 30 generations on Serratia Selection Plates (SSP) following methods outlined in Morran *et al.* (2011) (Fig. 1). Briefly, SSPs were constructed by spreading *S. marcescens* on 1/3 of the NGM-Lite (US Biological, Swampscott, Massachusetts, USA) agar of a 100 mm Petri dish, *E. coli* OP50 on the opposite 1/3 and ampicillin (200 μg mL^{-1}) in the middle 1/3 of the agar. *Caenorhabditis elegans* were plated onto the *S. marcescens* side of the plate and required to survive parasite exposure and travel to the *E. coli* side in order to reproduce and be passaged. The evolution treatment consisted of exposure to a static, non-evolving *S. marcescens* strain Sm2170, whereas the coevolution treatment consisted of exposure to coevolving *S. marcecens*, and control treatment groups were exposed to heat killed Sm2170. Host and parasite populations in the experimental coevolution treatment were passaged under the potential for reciprocal selection. Specifically, parasites were required to kill hosts for passage to the next round of selection, whereas hosts were required to survive parasite exposure and reproduce for their offspring to be passaged. This coevolution treatment differed from the evolution treatment in that within the evolution treatment, only the hosts were under selection to survive and reproduce. Importantly, *S. marcescens* strain 2170 served as both the ancestral parasite strain in the coevolution treatment and the static parasite strain in the evolution treatment. A subset of each experimental host population and coevolving *S. marcescens* was frozen and stored at −80 °C at generations 0, 10, 20 and 30.

Bacterial choice assay

Bacterial choice assays were performed using methods modified from Zhang *et al.* (2005) and Glater *et al.* (2014) (Fig. 2). Choice assays were set up in 100 mm petri dishes containing 30 mL of NGM-Lite. A 25 μL of *S. marcescens* (strain Sm2170 or coevolved) and *E. coli* were spotted onto opposite far sides of the plate and allowed to incubate at room temperature for 5 h. Host populations from the coevolution treatment were paired with their respective sympatric parasite population, which is the *S. marcescens* population with which they coevolved. Host populations were bleach synchronised and ~200 L4 individuals in M9 buffer were introduced onto the middle of the assay plate. Petri dishes were left ajar at room temperature for ~30 min until the M9 buffer had completely evaporated, at which point the lids were closed and dishes were moved into the 20 °C incubator.

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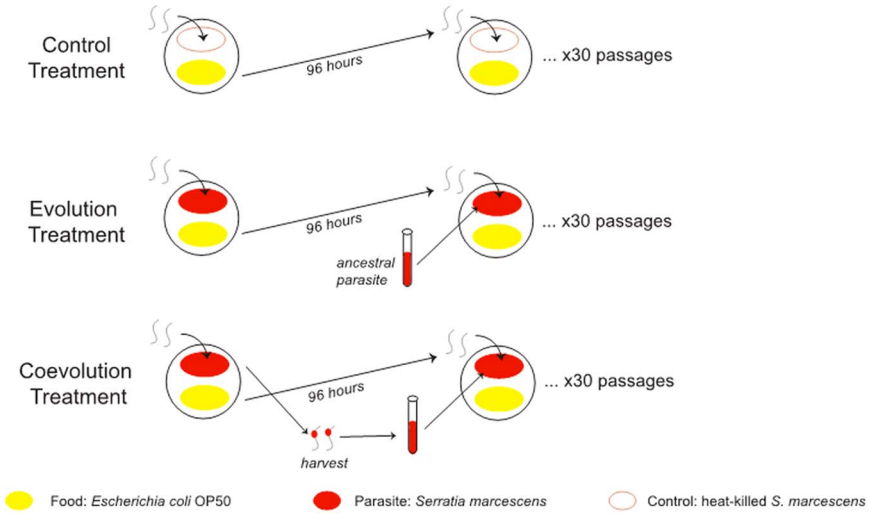


Fig. 1. Experimental evolution treatments. In a previous experiment, five replicate populations of mixed mating *Caenorhabditis elegans* hosts and five replicate populations of obligately outcrossing *C. elegans* hosts were passaged for 30 generations on three treatments: control, evolution and coevolution. For each replicate population, parental hosts were placed on the *Serratia marcescens* side of the Serratia Selection Plate. Then, after 4 days of parasite exposure, the host offspring were collected on the *Escherichia coli* portion and transferred to another plate. The hosts were exposed to heat-killed parasites in the control. The hosts were exposed to the same strain of live *S. marcescens* each generation in the evolution treatment. Live *S. marcescens* parasites were copassaged with the hosts in the coevolution. Parasites were extracted from dead hosts after 24 h of exposure and then used to seed the Serratia Selection Plate for the next generation of hosts.

Four hours after plating the hosts, assay plates were scored by counting the number of host individuals in each bacteria spot and on the rest of the plate. We assayed populations after 4 h because we observed that 4 h was the minimum amount of time required for >75% of the hosts to exhibit choice. Each choice assay was replicated six times.

Ancestral host populations, as well as host populations from the coevolution, evolution and control treatments were assayed for choice between *E. coli* OP50 and Sm2170. Further, host populations from generation 20 and generation 30 of the coevolution treatment were assayed for their avoidance of the ancestor parasite (Sm2170) and the generation 20 coevolved parasite. We assayed generation 20 hosts with their contemporary parasite from the coevolution treatment to assess parasite avoidance in the midst of the arms race. We assayed generation 30 hosts with their parasites from the recent past to determine if there is a time lag in the host response. We were unable to revive two of the five obligately outcrossing host populations from the coevolution treatment at generation 30 from frozen stock. Therefore, our analysis included only three replicate populations for this particular treatment by mating system combination.

The following equation, modified from Glater *et al.* (2014), was used to calculate the host bacterial choice index. We modified their equation by dividing the difference between the number of hosts on *S. marcescens* and the number on *E. coli* by the total number of hosts, rather than dividing the number of hosts that specifically chose either *S. marcescens* or *E. coli*. Thus, 'Total # hosts plated' indicates total number of hosts individuals assayed, regardless of where they were located upon scoring the assay. In each replicate assay, regardless of the host or parasite population, multiple host individuals were not in either bacterial spot at the end of the assay. Our method of calculating the bacterial choice index is a more precise measure of preference as a whole in that the absolute value of choice index scores are reduced by individuals that do not explicitly choose a bacterial spot. Further our method is more applicable in the context of our experiment because host fitness was dependent on both avoiding *S. marcescens* and feeding on *E. coli*, as opposed to only avoiding the parasite.

$$\text{Bacterial choice index} = \frac{(\#\text{ hosts in } S.\ marcescens - \#\text{ hosts in } E.\ coli)}{\text{Total}\,\#\,\text{hosts plated}}$$

A choice index >0 indicates a preference for the parasite *S. marcescens*. A choice index <0 indicates preference for *E. coli* (Fig. 2).

We performed analyses of variance (ANOVAs) in JMP 12 (SAS Institute, Cary, North Carolina, USA) on transformed mean bacterial choice index values for each replicate host population. Mean bacterial choice index values were square root transformed after adding a value of 1 to each mean to ensure that all values were positive prior to transformation. The transformed

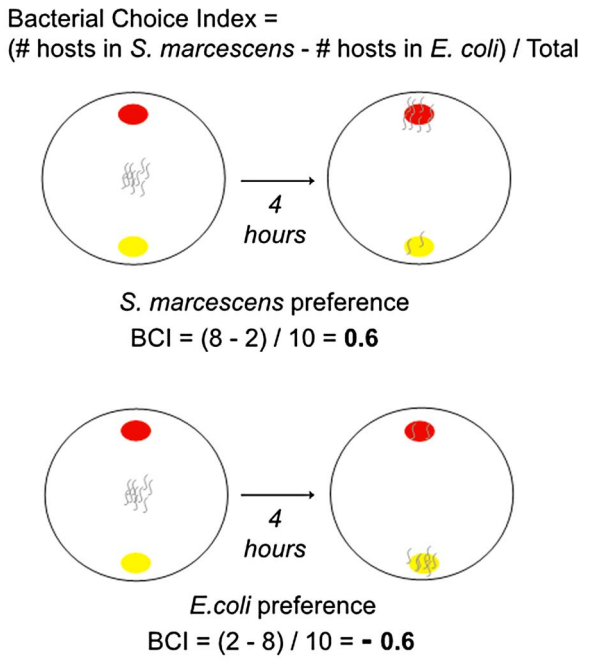


Fig. 2. BCI schematic. *Caenorhabditis elegans* hosts were placed between patches of *Serratia marcescens* and *Escherichia coli*, then their location was recorded after 4 h. These data were used to calculate the BCI for the hosts. BCI, bacterial choice index.

Table 1. Mixed mating host ancestral bacterial choice index table

Source	Sum of squares	D.F.	Mean square	*F*	*P*
Model	0·811	3	0·27	3·75	=0·0326
Error	1·15	16	0·072		
Total	1·96	19			

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mean values did not violate the ANOVA assumptions of normality and equal variances according to the Shapiro–Wilk and Levene's tests. We performed separate ANOVAs for the mixed mating (Table 1) and obligately outcrossing (Table 2) populations exposed to Sm2170. In both analyses, we tested the main effect of host treatment (ancestral, control, evolution and coevolution) on mean bacterial choice index values. We also used least squares mean contrast tests to assess differences between treatments within the mixed mating analysis. Then, we performed separate ANOVAs for the mixed mating (Table 3) and obligately outcrossing (Table 4) hosts from the coevolution and control treatments from generations 20 and 30 exposed to coevolved parasites from generation 20. In both analyses, we tested the main effects of host treatment (control and coevolution) and host generation (generations 20 and 30) and the treatment by generation interaction, on the transformed mean bacterial choice index values.

Results

Host bacterial food preference: ancestral and static parasite populations

We determined bacterial choice index scores for mixed mating and obligately outcrossing host populations presented with the choice between *E. coli* OP50 and the parasite strain Sm2170 (Fig. 2). Sm2170 served as both the ancestral strain to the parasite populations from the coevolution treatment and was also the static parasite strain for the evolution treatment throughout experimental evolution (Fig. 1). We assayed ancestral host populations and host populations after 30 generations of exposure to the control, evolution or coevolution treatments, for both the mixed mating and obligately outcrossing hosts. The mixed mating populations from the evolution and coevolution treatments exhibited significantly greater preference for *E. coli*, relative to the control populations (Fig. 3A and online Supplementary Fig. S1). Specifically, mean bacterial choice index scores were significantly reduced in mixed mating hosts from the evolution (Table 1 and Fig. 3A; $F_{1,16} = 4{\cdot}89$, $P = 0{\cdot}042$) and coevolution (Table 1 and Fig. 3A; $F_{1,16} = 9{\cdot}41$, $P = 0{\cdot}0074$) treatments, relative to control populations. Further, the populations from the evolution and coevolution treatments exhibited an overall mean preference for *E. coli*, as opposed to Sm2170 (Fig. 3A). However, we did not observe a significant change in bacterial preference (mean choice index scores) in the obligately outcrossing host populations, regardless of treatment (Table 2, Fig. 3B and online Supplementary Fig. S1). Therefore, mixed mating hosts evolved parasite avoidance behaviour in the presence of Sm2170, while obligately outcrossing hosts did not.

Host bacterial food preference: coevolved parasite populations

Next, we assessed host and parasite interactions from the coevolution treatment to determine if bacterial preference evolved during antagonistic coevolution. We measured bacterial choice index scores for mixed mating and obligately outcrossing host populations from the coevolution and control treatments at generations 20 and 30 of experimental evolution. Hosts were presented with a choice between *E. coli* OP50 and their sympatric parasite population, the specific parasite population with which the hosts were passaged, from generation 20 of coevolution. Host populations from both generations 20 and 30 of the experiment were tested to account for a potential time lag in the response of the hosts to the generation 20 parasites. Relative to controls, we observed no significant difference in the bacterial choice index scores of the mixed mating hosts (Table 3, Fig. 4A, online Supplementary Figs S2 and S3), nor the obligately outcrossing hosts (Table 4, Fig. 4B, online Supplementary Figs S2 and S3), in the presence of the coevolved parasites. Further all of the mixed mating and obligately outcrossing hosts exhibited a mean preference for the coevolved parasites after both 20 and 30 generations (Fig. 4). Therefore, at least after 20 generations of experimental evolution, hosts did not evolve parasite avoidance in response to their sympatric coevolving parasites.

Discussion

We found that some of the experimentally evolved populations of *C. elegans* evolved greater preference for *E. coli* when presented with a choice between *E. coli* OP50 and *S. marcescens*. This response to selection is particularly striking, given that *C. elegans* naturally prefer *S. marcescens* to *E. coli* (Zhang *et al.* 2005; Pradel *et al.* 2007; Glater *et al.* 2014). Our results suggest that this preference for *E. coli* is driven by active avoidance of *S. marcescens*, as opposed to increased preference for *E. coli*. The mixed mating hosts that were passaged with reciprocally evolving *S. marcescens* only exhibited *E. coli* preference in the presence of the ancestral Sm2170 strain (Fig. 3A), and not the coevolved populations of *S. marcescens* (Fig. 4A). Therefore, the hosts' evolved preference for *E. coli* is conditional on the parasite strain present and not a general increase in preference for *E. coli*. Nonetheless, the evolution of increased *E. coli* preference, as opposed to direct avoidance of *S. marcescens*, would also function as parasite avoidance in the context of our experiment (Morran *et al.* 2011). In the previous experiment, hosts were directly exposed to *S. marcescens* and crawled to *E. coli* to feed and reproduce (Fig. 1). Therefore, the evolution of host preference for *E. coli* would result in parasite avoidance as hosts exited the *S. marcescens* lawn to feed on *E. coli*. Regardless of the specific behaviour that evolved, we found that experimental evolution in the presence of either static or coevolving parasite populations resulted in the evolution of parasite avoidance in mixed mating host populations.

Interestingly, the mixed mating host populations that coevolved with *S. marcescens* populations exhibited parasite avoidance in the presence of the ancestral parasite populations (Fig. 3A), but did not avoid their contemporary coevolved parasites or coevolved parasites from the recent past (Fig. 4A). These results suggest that the coevolving parasite populations may have evolved to counteract avoidance by the host. The parasites in the coevolution treatment were required to kill hosts to gain fitness within the context of our experiment (Morran *et al.* 2011). Therefore, avoidance on the part of the host would be particularly costly for the parasites. It is perhaps not surprising that

Table 2. Obligately outcrossing host ancestral bacterial choice index table

Source	Sum of squares	D.F.	Mean square	*F*	*P*
Model	0·173	3	0·0577	0·95	=0·439
Error	0·971	16	0·061		
Total	1·144	19			

Table 3. Mixed mating host coevolved bacterial choice index table

Source	Sum of squares	D.F.	Mean square	*F*	*P*
Model	0·0001	1	0·0001	0	=0·9988
Error	0·1791	7	0·0256		
Total	0·1792	8			

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Table 4. Obligately outcrossing host coevolved bacterial choice index table

Source	Sum of squares	D.F.	Mean square	*F*	*P*
Model	0·098	1	0·098	2·44	=0·1567
Error	0·321	8	0·04		
Total	0·419	9			

parasite avoidance appears to have been negated when coevolving parasites had the ability to respond to the hosts. The parasite populations may have evolved a greater ability to attract the hosts to counteract avoidance by the hosts. However, the coevolved parasites are not more attractive to the control host populations (Figs 3A and 4A); therefore, any increased attractance would need to be very specific between sympatric hosts and parasites. This scenario is plausible, given that these coevolved parasite populations exhibited local adaptation with regard to their sympatric host populations (Morran *et al.* 2014).

In general, both the mixed mating and obligately outcrossing host populations evolved increased fitness in the presence of static and coevolving parasites after 30 generations of experimental evolution (Morran *et al.* 2011, 2014; Penley *et al.*, unpublished result). Here, we found that only the mixed mating populations evolved parasite avoidance as a mechanism of defence. However, avoidance cannot solely account for the increased fitness exhibited by mixed mating host populations from the coevolution treatment. These hosts evolved increased fitness in the presence of their coevolved sympatric parasites (Morran *et al.* 2014), yet did not exhibit avoidance behaviour in the presence of those parasites (Fig. 4A). Therefore, it appears that the mixed mating populations from the coevolution treatment evolved multiple mechanisms to facilitate adaptation to parasites. Our results suggest avoidance evolved early during experimental evolution, because the hosts exhibited avoidance in the presence of the ancestral parasites (Fig. 3A), but did not avoid contemporary coevolving parasites or coevolved parasites from the past (Fig. 4A). Therefore, another form or forms of host defence likely evolved during antagonistic coevolution to confer greater host fitness to the reciprocally evolving parasites.

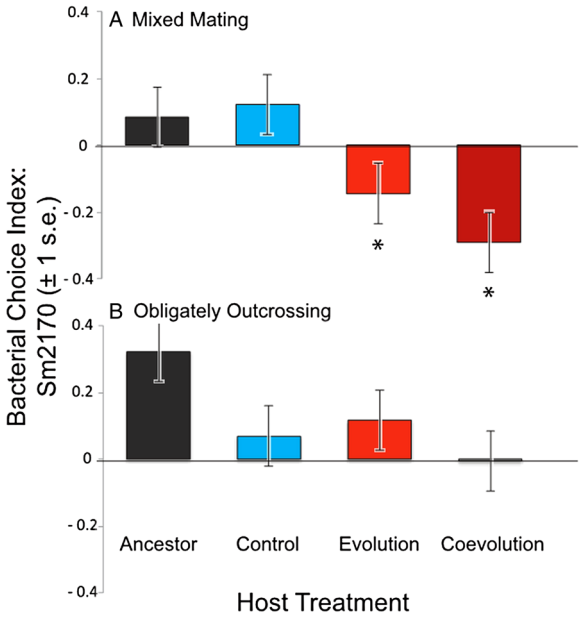


Fig. 3. Mean bacterial choice index scores on Sm2170 parasites. Positive choice index scores are indicative of host preference for *Serratia marcescens* Sm2170, while negative choice index scores indicate host preference for *Escherichia coli* OP50. (A) Mixed mating hosts from the evolution and coevolution treatments exhibit significantly increased preference for *E. coli*, relative to control and ancestral hosts; (B) obligately outcrossing host populations from the evolution and coevolution treatments do not exhibit altered preference relative to the control and ancestral host populations. Asterisks indicate significant difference from the control and ancestral populations.

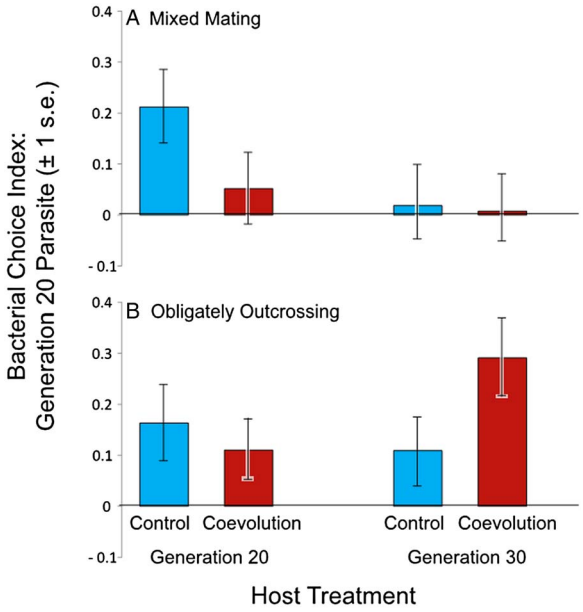


Fig. 4. Mean bacterial choice index scores on coevolved parasites. Positive choice index scores indicate preference for *Serratia marcescens* from the coevolution treatment after 20 generations of coevolution, while negative choice index scores indicate preference for *Escherichia coli* OP50. Coevolution treatment hosts were paired with their sympatric parasite population, while control treatment hosts were paired with the sympatric parasite population matching their respective coevolution treatment replicate population. (A) Mixed mating hosts from the coevolution treatment at generations 20 and 30 did not significantly differ in preference from control treatment populations at generations 20 and 30; (B) obligately outcrossing hosts from the coevolution treatment at generations 20 and 30 did not exhibit significant differences in bacterial preference relative to control populations.

In contrast to the mixed mating populations, the obligately outcrossing populations did not evolve avoidance behaviour to the parasites, regardless of the treatment (Figs 3B and 4B). This is somewhat surprising because we expect the efficacy of selection to be stronger in the obligately outcrossing populations (Hill and Robertson, 1966; Felsenstein, 1974; Hodgson and Otto, 2012), and thus their potential to evolve multiple mechanisms of defence could be greater than mixed mating populations. However, greater efficacy of selection in the obligately outcrossing populations may have simply favoured a different form of defence, like resistance or tolerance, which conferred greater overall fitness than avoidance behaviour. Given that the obligately outcrossing populations evolved greater fitness relative to the mixed mating populations (Morran *et al.* 2011, 2014; Penley *et al.*, unpublished result), this is a plausible scenario. Further work is required to identify the other mechanisms of defence that evolved during experimental evolution. Additionally, mapping the loci underlying the evolved defence traits may allow us to better discern the cause of the different evolutionary trajectories in the mixed mating and obligately outcrossing populations.

It is currently unclear why *C. elegans* tends to prefer a bacterial parasite relative to a generally benign food source, given that the dietary preference of *C. elegans* can evolve in response to selection from parasites. *Caenorhabditis elegans* may only rarely encounter virulent strains of *S. marcescens* in nature, so selection for avoidance could be quite weak. Conversely, natural populations of *C. elegans* may be adapted to local populations of *S. marcescens*

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and the small amount of laboratory assays conducted failed to detect this phenomenon. Few *C. elegans* strains have been tested for avoidance behaviour, while those tested were exposed to a very narrow subset of *S. marcescens* strains, so the current data available are not sufficient for detecting local adaptation. Indeed there is much yet to learn about *C. elegans* interactions with parasites in nature, and *C. elegans* ecology in general (Frézal and Félix 2015).

Despite the seeming discrepancy between natural populations of *C. elegans* and our experimentally evolved populations, experimental evolution is a powerful tool for determining what can happen in nature. Overall, we found that both the host mating system and host–parasite coevolutionary dynamics can significantly alter the interactions and reciprocal adaptations between the hosts and parasites. This work demonstrates that the specific traits that evolve to confer host defence are not necessarily uniform for a particular host–parasite interaction. Instead, different host populations, or perhaps even different hosts within the same population, can exhibit varying forms of host defence against the same parasite. Although many factors likely contribute to the large variety of host defence mechanisms that exist in nature, we found that the host mating system and evolutionary history with parasites can shape the evolution of specific defence mechanisms within a host population.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182017000804.

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