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LETTERS

Host–parasite 'Red Queen' dynamics archived in pond sediment

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Antagonistic interactions between hosts and parasites are a key structuring force in natural populations, driving coevolution[1,2]. However, direct empirical evidence of long-term host–parasite coevolution, in particular 'Red Queen' dynamics—in which antagonistic biotic interactions such as host–parasite interactions can lead to reciprocal evolutionary dynamics—is rare[3–5], and current data, although consistent with theories of antagonistic coevolution, do not reveal the temporal dynamics of the process[6]. Dormant stages of both the water flea *Daphnia* and its microparasites are conserved in lake sediments, providing an archive of past gene pools. Here we use this fact to reconstruct rapid coevolutionary dynamics in a natural setting and show that the parasite rapidly adapts to its host over a period of only a few years. A coevolutionary model based on negative frequency-dependent selection, and designed to mimic essential aspects of our host–parasite system, corroborated these experimental results. In line with the idea of continuing host–parasite coevolution, temporal variation in parasite infectivity changed little over time. In contrast, from the moment the parasite was first found in the sediments, we observed a steady increase in virulence over time, associated with higher fitness of the parasite.

It is notoriously difficult to study evolutionary dynamics in nature because time series over many generations are needed. Laminated lake sediments, however, provide a unique possibility of reconstructing the evolutionary dynamics of natural populations over time, because many aquatic organisms produce dormant propagule banks that archive gene pools. This approach has already revealed adaptive microevolution in natural populations of *Daphnia* in response to changes in predation pressure[7,8] and anthropogenic perturbation[9].

Although theory suggests that host–parasite interactions impose frequency-dependent selection, leading to Red Queen dynamics, empirical data on temporal dynamics in natural settings are entirely lacking[3,6]. *Daphnia* and its microparasites show characteristics that are expected to lead to strong coevolutionary responses: there is ample genetic variation in *Daphnia* resistance to parasites[10], host–parasite interactions are genotype specific[11,12] and the genetic structure of *Daphnia* populations shifts during epidemics[13]. There is also evidence of local parasite adaptation[14] and short-term parasite-mediated selection in *Daphnia*[15,16], which affects host–parasite dynamics[17]. Yet it has so far remained impossible to document the long-term dynamics of *Daphnia*–parasite coevolution in a natural setting[6]. The fact that both *Daphnia* and its parasites produce dormant propagule banks[18], which accumulate in pond sediments, offers the opportunity to address this gap.

We sampled two sediment cores from a shallow pond (Oude Meren 2, 'Abdij van 't Park', Heverlee, Belgium[18]) where *Daphnia magna* coexists with the bacterial endoparasite *Pasteuria ramosa*. From different sediment layers of these cores we hatched *D. magna* clones from dormant eggs and picked up isolates of *P. ramosa*. Each depth represents a snapshot in the arms race of these antagonists, corresponding with a historical time fragment of the parasite and the *Daphnia* population. The oldest layer studied here (deepest depth 24 cm, a maximum of about 39 years old) represents the first time that both *D. magna* and *P. ramosa* co-occurred in this pond[18]. In two cross-infection experiments, we exposed *Daphnia* clones from eight (experiment 1) or seven (experiment 2) depths to parasite isolates from the next layer down, the same layer and the next layer up. Thus, the host was exposed to 'past', 'contemporary' and 'future' parasite isolates (further referred to as a time shift of parasites relative to host populations).

The reciprocal nature of Red Queen dynamics means that they are not always easily visible[6], because the fitness of both antagonists may change only slightly over time[3]. Indeed, Red Queen dynamics may result in no or few apparent changes in the phenotypes of the interacting populations, even though there may be continuous changes in the underlying genetic structure. The Red Queen hypothesis has received much attention because it implies that the host benefits by producing genetically heterogeneous offspring by means of sexual reproduction, thus creating new defence mechanisms against fast-evolving parasites[19,20].

Our results show that over a timescale of about two to four years (the temporal resolution of our sediment layers), parasites tracked common host clones. On average, infectivity was higher when *Daphnia* were exposed to contemporary (average infectivity 0.65) parasites than to parasites from previous (average infectivity 0.55) growing seasons (Fig. 1). However, parasite adaptation was quickly lost, because average parasite infectivity was lower when *Daphnia* clones were confronted with future parasites (average infectivity 0.57) than with contemporary parasites (Fig. 1). There was a significant time-shift effect ($P = 0.05$) as well as a very significant depth × time-shift interaction (Table 1). If past, contemporary and future parasites are designated P, C and F, respectively, the consistency of the pattern with the highest infectivity in the contemporary combinations across depths was high ($P < C$ in 10 of the 13 depths tested, and $C > F$ in 11) but was different in two depths (D5 and D7 from experiment 2; Fig. 1), generating the overall clone-depth × time-shift interaction (Table 1). The time-shift effect remains significant when considering only periods in which multiple parasites were examined (experiment 1, $P < C$ in six of the seven depths tested, and

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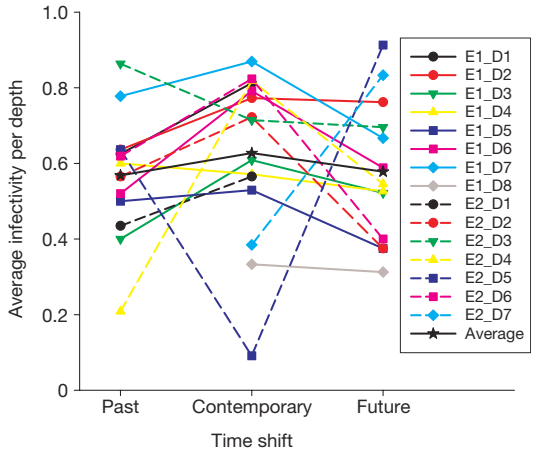

Figure 1 | Experimental results on temporal parasite adaptation. Average proportion of infected hosts when confronted with 'past', 'contemporary' and 'future' parasite isolates. Black stars, mean infectivity.

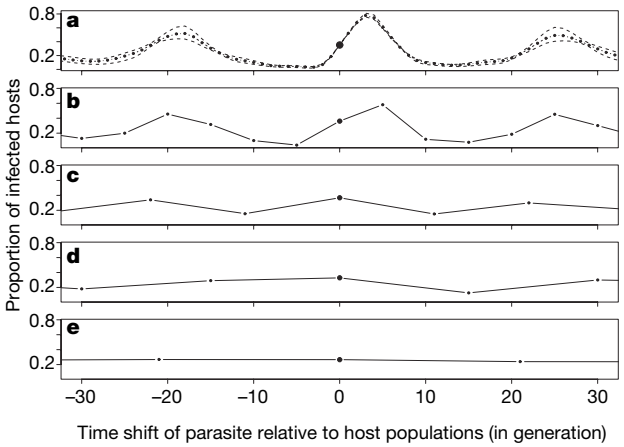

Figure 2 | Parasite infectivity as a function of the time shift of the parasites relative to the host population in the model simulation. Parasite infectivity averaged over 1 (**a**) 5 (**b**), 11 (**c**), 15 (**d**) and 21 (**e**) generations. A negative time refers to parasites from the past. Scatters around the line represent the 95% confidence interval. The compatibility matrix of parasite and host genotypes is defined by a matching allele model (MAM) with one locus and four alleles (Supplementary Information).

C > F in seven; goodness of fit (deviation/degrees of freedom) = 1.293; time-shift effect $P = 0.01$; contrast analyses: C − P, odds ratio 1.869, $P = 0.0145$; C − F, odds ratio 1.71, $P = 0.036$). There was no significant time-shift effect when considering only periods in which single parasites were examined (experiment 2); instead, there is a very significant clone depth × time-shift interaction in this experiment (experiment 2, P < C and C > F in four of the six depths tested; goodness of fit (deviation/degrees of freedom) = 1.206; interaction $P < 0.0001$), which we suspect reflects the large variation that occurs among parasite isolates and that is misattributed to a clone depth × time-shift interaction when only one parasite is considered at each depth (Supplementary Information).

This rapid tracking of host genotypes by parasites is unlikely to be driven by strong continuous immigration of novel genotypes into the population (see Supplementary Information). We therefore conclude that our study reveals fast evolutionary changes, with parasites adapting to infect contemporary host genotypes. The loss of parasite infectivity in later years may be driven by parasites adapting to the changing host population. Often, when parasites adapt to different host genotypes, loss of adaptation to former hosts is seen[21]. These observations are consistent with a model of negative frequency-dependent selection. We tested the consistency of our experimental results with a haploid parasite and diploid host coevolutionary model based on a matching allele interaction matrix (see Supplementary Information). Simulations of this model resulted in negative frequency-dependent selection. Using realistic parameter settings for our host–parasite system, we found that parasite isolates from the near future were the most infective (Fig. 2a, b) and that hosts were least infected when exposed to parasites from the recent past. Allotemporary combinations farther into the future or the past showed a cyclic pattern of infectivity (Fig. 2a, b). This pattern changes when the measure of parasite infectivity is averaged over several generations, as in our experiment, in which hosts and parasites were pooled over 2-cm sediment layers (which may contain 10–20 *Daphnia* generations). This yields a pattern in which contemporary parasites are more infective than past and future parasite isolates, which is consistent with our results (Fig. 2c). As expected, any further averaging causes a loss of dynamics (Fig. 2d, e). The dynamics seen in the model are qualitatively robust to various parameter settings: with more alleles the peaks spread out, whereas they become flatter when virulence is reduced (Supplementary Figs 2 and 3). Higher mutation rates tend to promote faster adaptation and result in smoother adaptation dynamics (Supplementary Fig. 4).

Table 1 | Results of logistic regression on infectivity

Parameter	Degrees of freedom	Residuals of deviance	Residuals of degrees of freedom	Deviance	*P*
Null	–	–	844	1,143.56	–
Time shift	2	5.89	842	1,137.67	0.05
Experiment	1	0.20	841	1,137.47	0.65
Clone depth	7	11.61	834	1,125.86	0.11
Clone depth × time shift	12	57.44	820	1,063.11	<0.0001

A binomial model, logit link, was used, with time shift reflecting the difference between the combinations of host clone and parasite isolate depth (three levels: 'past', 'contemporary' and 'future'), excluding non-significant interactions.

Our results are consistent with studies on mechanistic aspects of coevolution in the same host–parasite system[10,11,22]. Furthermore, it has been shown that *Pasteuria* epidemics can select for resistance in *Daphnia*[23] and that genetic variation as observed under laboratory conditions explains disease occurrence under natural conditions[24]. Our model, which was streamlined to match the details of our experimental design, indicates that our results are consistent with antagonistic coevolution on the basis of a matching-allele-type assumption. However, the model also shows that temporal adaptation differs strongly in strength and in cycle length when certain aspects of the host–parasite system are altered. Such changes are crucial in relation to the experimental design, because different time shifts or thicknesses of sediment layers (see Fig. 2 and Supplementary Fig. 2) could make it more or less likely that the pattern seen in our study will be found (Fig. 1). Indeed, an earlier one-year time-shift study employing a different design and working at a different location was unable to find evidence for coevolution[25]. Furthermore, the arms race may be disguised altogether under more complex ecological conditions, for example, where other antagonists, such as predators, have a dominant impact[26]. Our analysis also reveals that some of the contrasts between past and contemporary time layers and between contemporary and future layers deviate from the average picture (Fig. 1 and Supplementary Fig. 5). At certain times, one or the other antagonist may lag in the arms race. All these factors highlight that the signature of antagonistic coevolution in natural populations may be found only in host–parasite systems with certain properties and may need experimental conditions well chosen to match the biology of the system.

The continuous time series from the sediment cores did not reveal a net change in parasite infectivity over time (Fig. 3a, d). However, we found evidence for gradual changes in other components of parasite fitness. Starting from the first record of the parasite (deepest depth 24 cm), production of the *Pasteuria* transmission stages increased with isolates of more recent origin (Fig. 3b, e). Increased parasite

spore production was associated with stronger fecundity reduction in the *Daphnia* host (product moment correlation between spore production and fecundity reduction: $n = 15$, $R = 0.98$, $P < 0.0001$; see also ref. 27). Thus, the parasite decreased host fecundity progressively over time (Fig. 3c, f).

This increase in spore production and virulence in *Pasteuria* over time (but not its infectivity; Fig. 3) may reflect adaptation of the parasite to the host. It is unlikely to be explained by differences in the duration of dormancy of the parasite spores (as a result of genetic deterioration or trade-offs between dormancy and other traits, for example). All parasite spores were revived by a growth cycle in *Daphnia* hosts after they were picked up from the sediment. Consequently, all spores used in the experiment were physiologically of the same age. Moreover, *Pasteuria* that were randomly isolated with a standard set of host clones did not show an increase in spore production over time[18] (results not shown). Furthermore, it is unlikely that endospores such as those from *Pasteuria* accumulate heritable damage that is reflected in fitness parameters within the time span covered in our sediment core (oldest layer 24 cm). Bacilli, which are closely related to *Pasteuria*, protect the DNA of their endospores with small acid-soluble proteins during dormancy[28]. An absence of genetic degeneration during dormancy has been reported in plant seeds up to 150 years old[29]. At present it is not clear what mechanism can explain the different dynamics in parasite spore production and virulence compared with infectivity.

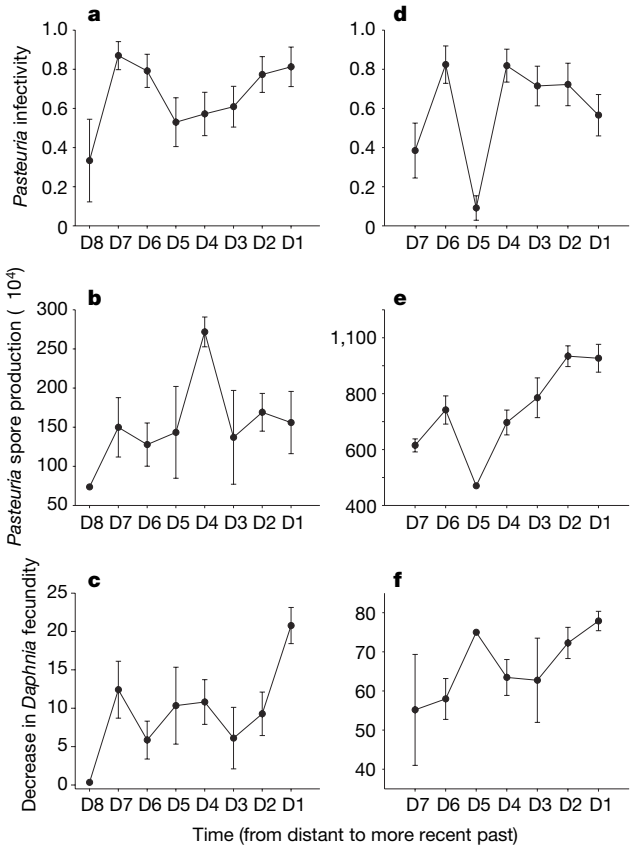

Figure 3 | *Pasteuria* infectivity and virulence over time. a–c, Experiment 1; **d–f**, experiment 2. **a**, **d**, *Pasteuria* infectivity; **b**, **e**, *Pasteuria* spore production; **c**, **f**, decrease in *Daphnia* fecundity (clonal average of control individuals minus clonal average of infected individuals). Results are means ± s.e.m. and include only 'contemporary' combinations. Time reflects parasite isolate depth, with D8 referring to the oldest sediment layer. The effects of parasite isolate depth as continuous covariable in a general linear model (including significant effects of experiment; parasite isolate depth was nested in experiment) are as follows: on *Pasteuria* infectivity, $F_{2,108} = 0.52$, $P = 0.59$; on *Pasteuria* spore production, $F_{2,80} = 9.6$, $P = 0.0002$; on decrease in *Daphnia* fecundity, $F_{2,81} = 5.03$, $P = 0.009$.

It is likely that different genes contribute to different parasite fitness components and thus follow different evolutionary dynamics.

In this historical reconstruction of host–parasite interactions in a natural population, we provide empirical evidence for coevolution based on fluctuating selection. Such Red Queen dynamics have been suggested to have a role in widespread biological phenomena such as the evolution and maintenance of sexual reproduction[19,20] and the maintenance of genetic polymorphism at disease loci[30]. In this sense, antagonistic coevolution may have a key role in the evolution of biodiversity on Earth[2].

METHODS SUMMARY

Daphnia host clones and parasite isolates were derived from two sediment cores (one per experiment) that were sliced into depth increments. From each core, eight 2-cm depth increments were used, covering a time interval of about 17 to 28 years—thus, about 2–4 years per 2-cm depth—with the age of the deepest sediment layer being about 29–39 years. On the basis of the similar profile of the number of dormant eggs in the different depths of the cores of both experiments (Supplementary Fig. 1), we numbered the depths used equally between the cores. Host clones were obtained through hatching of dormant eggs from each depth. Parasite isolates were obtained by exposing a random set of *Daphnia* clones to sediment from each depth. For experiment 1, we obtained three parasite isolates per depth (eight depths × 3 = 24 in total). For experiment 2, we obtained only one parasite isolate per depth from the first seven depths. After controlling for maternal effects, neonates of the host clones were isolated and individually exposed to a parasite or a control treatment. In the parasite treatments, the host clones were exposed to 'past', 'contemporary' and 'future' parasite populations (referred to as time-shift treatment). Therefore, for each depth, three (experiment 1) or eight (experiment 2) host clones were exposed to three (experiment 1) or one (experiment 2) parasite isolate(s) from the next layer down, the same layer and the next layer up, in three replicates. During the experiments, the number of juveniles produced and the time to castration (when *Pasteuria* prevents *Daphnia* from reproducing) was recorded. After 26 days (experiment 1) or 23 days (experiment 2), all *Daphnia* were checked for infection and *Pasteuria* spore production was recorded.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Sediment cores. For experiment 1, a core with a diameter of 5 cm was taken in October 2002 and was sliced in depth increments of 2 cm. For experiment 2, a core with a diameter of 15 cm was taken in January 2006 and sliced in increments of 1 cm. The outer 1 mm of each slice was removed. Dating of the core was performed on the basis of the assumption of constant sedimentation rates in terms of sediment mass (see refs 31, 32). Because the time axis should be considered indicative, only rough statements with respect to time can be made.

***Daphnia* clones and parasite isolates.** Host clones were collected by hatching dormant eggs that were isolated from ephippia. Hatching was induced at 15 °C under a 16 h:8 h light/dark cycle. In all further experiments, *Daphnia* clones were kept under standardized culture conditions (19 ± 1 °C and a light/dark cycle of 16 h:8 h) and fed daily with the alga *Scenedesmus acuminatus*.

The spores of the parasite isolates of the cross-infection experiments were isolated by exposing a random set of *Daphnia* clones (16 clones in experiment 1 and 20 clones in experiment 2, hatched from all sediment depths) to sediment (including *P. ramosa* spores) from each depth separately. Two replicates of each clone were individually exposed to an equal amount of sediment from all depths in 50-ml jars (4×10^4 algal cells ml^{-1} per day). After 28 days of exposure all infected *Daphnia* were isolated for use as a source of spores for the parasite isolates of the cross-infection experiment. For each sediment depth, each parasite isolate was isolated from a different clone. To obtain a sufficient number of spores from each parasite isolate in the cross-infection experiments, all parasite spores were propagated once in *Daphnia* juveniles from the same clone as the *Daphnia* mother from which the spores were originally isolated. Spore solutions, obtained from one 'squashed' infected mother, were divided between two (experiment 1) or three (experiment 2) 50-ml experimental jars with juveniles. For experiment 1, we obtained 24 parasite isolates in total, three from each 2-cm depth (eight depths × 3 = 24). In experiment 2, we lost several isolates during the propagation phase and finally obtained only seven parasite isolates in total, one from each of the first seven 2-cm depths. For each parasite isolate, infected *Daphnia* were then isolated, pooled and ground up to prepare final spore solutions for the cross-infection experiment. Parasite isolates from each depth were independent from the host clones used in the cross-infection experiment because, in general, the *Daphnia* clones that picked up the parasite spores originated from a different depth from the host clones in the cross-infection experiment. Spore solutions and the amount of added *Daphnia* tissue were standardized across all parasite isolates in each final cross-infection experiment.

Cross-infection experiments. Maternal lines were started up with adult female *Daphnia* from clonal stock cultures kept for several generations in the laboratory and derived from a single dormant egg. Neonates of the second clutch of the second-generation maternal lines were isolated and individually exposed to a parasite or control treatment in 100-ml (experiment 1) or 50-ml (experiment 2) jars. In experiment 1, whenever possible, three *Daphnia* clones (other than those used to pick up the parasite isolates) from each 2-cm depth were exposed to nine parasite isolates (three parasite isolates from the next layer down, the same layer and the next layer up) in three replicates. Only two parasite isolates were used for depth five and only one for depth eight. In all parasite treatments, 2.5×10^5 spores ml^{-1} were added on day one, and 2.5×10^4 spores ml^{-1} on day seven. For experiment 2, we did not obtain as large numbers of spores as in the first experiment, and we used a lower spore concentration in this experiment. In experiment 2, whenever possible, eight *Daphnia* clones from each 2-cm depth were exposed to three parasite isolates (one parasite isolate from the next layer down, the same layer and the next layer up) in three replicates. We did not obtain parasite isolates from the lowest depth in experiment 2; E2_D8 was therefore not included in the analyses. In all parasite treatments, 1.3×10^3 spores ml^{-1} were added on day 1, and 0.65×10^3 spores ml^{-1} were added on day 3. In experiment 1, all *Daphnia* were fed daily with 0.8×10^5 algal cells ml^{-1}; in experiment 2, they were fed with 0.5×10^5 algal cells ml^{-1} for the first six days, and afterwards with 1.5×10^5 algal cells ml^{-1}. Food levels were relatively equal (difference of 0.3×10^5 algal cells ml^{-1}) and low in both experiments in the first days of the experiment, to ensure that parasite uptake would be high. In comparison with experiment 1, food quantity in the second part of experiment 2 was considerably higher. This was to ensure that our previous patterns could also be found when the *Daphnia* were not stressed. The medium was refreshed every time the host released a clutch, or at least every fourth day.

Data analysis. In the infectivity analysis we were interested in the effect of host clones and the origin of parasite isolates. We tested whether infectivity in contemporary associations was higher than in hosts exposed to future or past parasites. We performed a logistic regression on infectivity in R[33]: binomial model, logit link. Factors were time shift (time difference between the different depth combinations past, contemporary and future), experiment and clone depth (nested in experiment). Non-significant interactions were excluded. When we included host clone in the model as a random effect, our results on time shift did not differ.

In the virulence analysis we were interested in whether there was an evolutionary change in *Pasteuria* spore production and virulence (estimated as parasite-induced decrease in host fecundity and earlier time to castration (when *Pasteuria* prevents *Daphnia* from reproducing)) over time. We only included contemporary associations. General linear models with time shift and experiment as categorical factors and parasite isolate depth (nested in experiment) as a continuous factor (there were no parasite isolate effects) were performed on the averages for each combination of host clone and parasite isolate. Infection rate was included as a continuous covariable in the general linear models of the *Pasteuria* spore production and virulence parameters. There was no interaction between parasite isolate depth and time shift in any of the parameters tested.

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