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ORIGINAL RESEARCH

Influence of co-evolution with a parasite, *Nosema whitei*, and population size on recombination rates and fitness in the red flour beetle, *Tribolium castaneum*

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Abstract The high prevalence of meiotic recombination—an important element of sexual reproduction—represents one of the greatest puzzles in biology. The influence of either selection by a co-evolving parasite alone or in combination with genetic drift on recombination rates was tested in the host-parasite system *Tribolium castaneum* and *Nosema whitei*. After eight generations, populations with smaller genetic drift had a lower recombination rate than those with high drift whereas parasites had no effect. Interestingly, changes in recombination rate at one site of the chromosome negatively correlated with changes at the adjacent site on the same chromosome indicating an occurrence of crossover interference. The occurrence of spontaneous or plastic changes in recombination rates could be excluded with a separate experiment.

Keywords *Tribolium castaneum* · *Nosema whitei* · Recombination · Sex · Genetic drift · Red Queen · Hill-Robertson

Introduction

Theoretical studies have often shown that meiotic recombination can evolve only under a very limited set of conditions (Barton and Otto 2005; Otto and Barton 2001). Empirically, the population-wide frequency of recombination can be changed by selection as demonstrated by many independent studies (Korol 1999; Rice 2002). In fact, numerous genes and loci have been identified that underlay the physiological and molecular processes of meiosis and recombination (Brooks 1988), which are collectively labelled recombination modifiers. Genetically, the only effect of recombination is to break existing linkage disequilibria (LD) in a population. Hence, any adaptive theory for recombination must explain why breaking down existing LD is beneficial. Theory suggests that breaking LD has a short- and a long-term effect (Barton 1995; Salathé et al. 2008). The long-term effect is to increase genetic variance in the population, which allows the response to selection to be swifter (Burt 2000). The short-term effect acts on the next and following generations (delayed immediate effects) and depends on whether good or bad combinations of alleles are generated (Salathé et al. 2008).

It has been suggested that directional selection combined with genetic drift can cause a recombination modifier to spread. The reason is that in this case, the combined effects of these two factors tend to generate negative LD more often than positive LD (the Hill-Robertson effect; see (Otto and Barton 2001) for details). Per definition, negative LD is found when beneficial alleles are located in genetic backgrounds that are less fit than average (i.e. the combination of alleles runs counter to the currently best combinations); vice versa, positive LD can be defined likewise (Otto and Barton 2001). Negative LD in turn is beneficial to the spread of recombination. A second major contender to explain the adaptive value of recombination is the process of fluctuating epistasis generated by antagonistic co-evolution with rapidly evolving parasites (the "Red Queen hypothesis"). Models show that this scenario also

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provides a powerful selection regime that can favour the spread of recombination modifiers, and under a wider range of conditions than previously thought (Peters and Lively 1999; Salathé et al. 2008).

Whereas the reasons that recombination can spread in populations has received a lot of theoretical attention, there is a deplorable lack of empirical studies, especially experimental studies, addressing the hypothesized effect of host-parasite co-evolution. In the only existing explicit test so far, experimental co-evolution of the red flour beetle, *Tribolium castaneum*, with its natural microsporidian parasite, *Nosema whitei* (Weiser), did indeed lead to an increase in the population-wide recombination rate as compared to controls and those populations exposed to directional selection by an insecticide (Fischer and Schmid-Hempel 2005). *N. whitei* infects larvae and causes drastically increased mortality in larvae and reduced fecundity in adults (Armstrong and Bass 1986). Spores contained in the flour are ingested by the growing beetle larvae and induce the infection. Infection also occurs when dead larvae are cannibalized by others. Here, we will expand on this lead by scrutinizing the effect of antagonistic co-evolution in combination with differences in population sizes (large or small populations). Based on previous experience, the experiment was run for up to eight generations. Similar to the earlier study, recombination rate was measured before and during the experiment across two different intervals on each of two chromosomes, taking these four intervals as tokens for the genome-wide recombination rate. In addition, we measured host fitness by the number of offspring produced (fecundity) for each of the four treatment groups. Recombination rates were expected to increase in co-evolved populations according to the Red-Queen hypothesis, or in small (co-evolving) populations according to the drift model. In an auxiliary experiment we tested furthermore, whether recombination rates may change plastically (i.e. within one generation) in response to exposure to parasites.

Methods

Eleven lines of *T. castaneum* were obtained from the USDA Grain Marketing Research Laboratory (R. Beeman), and from three laboratories in Germany (R. Schröder at the University of Tübingen, J. Trauner at the University of Erlangen, and G. Bucher at the University of Göttingen). The different lines were kept separately in the lab for some time and then, explicitly, for two generations in preparation of the experiment that started in autumn of 2005. Subsequently, each stock line was split into two small populations with 50 beetles, and two large populations with 500 beetles each (four sub-populations in total). One small and one large population were paired to serve as (non-infected) controls whereas their counterparts were also paired (small, large populations) to serve as the treatment group infected with *N. whitei*. Hence, from the original 11 beetle lines a total of 44 populations (22 large and 22 small, half of those infected and half controls) were thus created, representing 11 replicate lines for each of the four treatment groups of the experiment. Population sizes of 50 and 500 were chosen according to practicability and as informed by the numerical simulations done by (Otto and Barton 2001). Furthermore, the design of using 11 different lines separately each meets the postulated requirement that populations start with different LD's to reduce the possibilities that all start with the same genetic associations (Otto and Barton 2001). Beetles were kept on yeast-enriched flour (5% yeast) at 33°C and 80% r.H. in plastic jars. Small populations were provided with 20 g of flour, large populations with 200 g per jar such that the amount of flour was equalized for the two population sizes, i.e. the same amount of flour was available per beetle. In order to get distinct generations, beetles were allowed to lay eggs for 5–7 days into the medium before being removed. The eggs and larvae were then left to develop and after approximately 40 days (during which no reproduction took place), 50 (small populations) or 500 (large populations) of the subsequently hatched and surviving beetles were transferred onto new medium in a new jar as breeders for the next generation. Virtually no mortality at the larval stage was observed in non-infected populations, suggesting that the parasite effects were the major source of mortality. The experimental passaging of hosts and parasites over generations inevitably generates serial bottlenecks, as appropriate for mimicking the effect of drift.

At the start of the experiment, five out of eight stocks of *N. whitei* (extracted from eight beetle lines outside of the experiment) were randomly chosen and admixed to the flour of the respective beetle population as a starting inoculum. Each infected beetle population (replicate line) received a different set (as given by the sets of five out of eight combinations) of parasite-lines. In each infected beetle population, the potential for ongoing co-evolution of parasites and hosts was ensured by infecting the next generation of beetles of the same experimental replicate population. At the beginning of the experiment, the spore-concentration was 10^3 spores/g flour but had to be altered to 2×10^4 spores/g flour in later generations (after generation 5, to 10^5 spores/g flour) in order to balance the increase in resistance and thus to get enough spores infecting the next generation at a sufficient rate; in the process, the concentration was always kept the same for large and small populations in a particular generation, i.e. this increase was balanced over all treatments and replicates.

Recombination frequencies were assessed in females only, at the start of the experiment (in stock lines) and in the generations 4 and 8: Estimates were obtained by a classical backcross of experimental females to homozygous recessive marker males as in (Fischer and Schmid-Hempel 2005). For this purpose, three experimental females per population were each singly mated with a marker male bearing mutations for phenotypic traits on linkage group nr.1 (with recessive markers plt, py, pd); another three females per beetle population were mated with a marker male bearing mutations for phenotypic traits on linkage group nr.2 (with recessive markers ub, pas, apt) (i.e. a total of six females that each contributed two measures, i.e. two intervals per linkage group). The physical positions of these markers on the genome are fixed. The genetic distance between two given markers on a linkage group can be estimated by the frequency of recombination events between the two markers and converted into centiMorgans [cM]. The distances of our markers under standard conditions were as follows (Fischer and Schmid-Hempel 2005): plt-py, 18.0 cM; py-pd, 9.0 cM; ub-pas, 16 cM; pas-apt, 4.7 cM. To make these markers visible, female offspring (F1) of a given "experimental female" × "marker male"-cross were raised under control (parasite-free) conditions, and subsequently backcrossed with marker males bearing the same mutations as those of the female' father. The resulting F2-offspring were again raised under control conditions and a total of 50 offspring beetles for every F1-female were analyzed for the frequencies of parental or recombinant phenotypes by visual inspection under the stereo microscope. Hence, the individual F2-offspring carried particular combinations of markers that were characteristic for either parental or recombinant type; thus, actual recombination rate is measured in F1-females produced by the females taken from the experimental populations. In total, about 30'000 beetles were scored for markers and the resulting recombination estimated. In generation 4 and 8, the total number of F2-offspring produced was recorded and served as a measure of fitness of F1-females. For practical reasons, this was done for F1-females that were crossed with marker males carrying mutations on linkage group nr.2 (i.e., for half of the F1-females). For practical reasons, too, in generation 4, F1-females were allowed to lay eggs for 13 days, and in generation 8 for 10 days. Fitness measures will thus only be compared within but not between generations. Recombination rates were furthermore checked for crossover interference, i.e. for statistical associations between different intervals over which recombination was measured. For this purpose, differences in recombination rate for each site between generation 0 and 4, between generation 4 and 8, and between generation 0 and 8 were calculated. The differences for the two adjacent sites on linkage group nr.1 and on linkage group nr.2 were then tested for correlations.

Data were analyzed with SPSS v. 11 for Macintosh. For the statistical analysis, the average recombination rate of the four intervals on the genome was calculated. In each generation, three measurements of recombination rate (from offspring of one female each) for linkage group nr.1, and three measurements for linkage group nr.2 existed per beetle line; we focused on the average recombination rate of all six measurements (females) in the statistical analysis. Similarly, the fitness measurements of the three replicate females (only those belonging to linkage group nr.2 were used, see above) were averaged. In all analyses, population size (large, small) and parasites (control, co-evolved) were fixed factors, beetle line (11 lines) was a random factor. ANOVAs were performed separately for generation 4 and 8 both for recombination rate and fitness; in addition, a repeated ANOVA with generation as within-subject factor was conducted for recombination rate. All analyses were done with two-tailed probabilities. Unless specified otherwise, data are given as mean ± 1 S.E. Note that we used random factors in mixed model ANOVA, which yields fractional d.f.

Finally, a separate experiment was set up to test for the possibility of plastic changes of recombination rates, i.e. up-regulation after infection. For this, five lines of *T. castaneum* were obtained from three different laboratories in Germany ("wild types") and cultivated under standard conditions (as discussed above). Similarly, recombination frequencies were measured by crossing and back-crossing wild-type females with the marker males as above (with two females per line). Per parental female, one control and one infected F1-female were backcrossed with the respective mutant male. Again, a total of 50 beetles of the resulting F2 offspring per F1-female were analyzed afterwards for the frequencies of parental or recombinant phenotypes. Using this crossing scheme, recombination rate was therefore again measured in F1-females. In order to measure plastic responses towards parasites, it was therefore also necessary to infect F1-females, for which we used again *N. whitei*. From each parental mating pair ("wild-type"-female x mutant-male) eggs were collected and split equally into two portions. One half of the eggs were raised on parasite-free flour under standard conditions (yielding the control F1). The other half of the eggs was reared under standard conditions on flour where a sublethal dose of 3.33×10^2 spores/g flour from a single strain of *N. whitei* had been admixed (yielding the parasitized F1). Recombination was then measured in the offspring (F2) of these F1-mothers. A total of five different strains of *N. whitei* (extracted from five external beetle lines) were used in the experiment. F2-beetles were raised on parasite-free flour under standard conditions. Data were analyzed with SPSS,

v. 11 for Macintosh. As before, for the statistical analysis, the average recombination rate of the four intervals (two intervals on each of two linkage groups) was calculated for each of two control females and each of two infected females per beetle line (totalling 5 lines × 2 beetles = 10 beetles per treatment) and analyzed with a paired T-test (two-tailed probabilities).

Results

Recombination rate (the estimated average of four genomic intervals in three replicates from the ANOVA) in the stock lines before the start of the experiment was 11.9 ± 0.533%. In generation 4, estimates were made for small (11.8 ± 0.503%) and large (11.8 ± 0.404%) controls, and for small (11.2 ± 0.472%) and large (12.0 ± 0.255%) infected (co-evolution) lines. For generation 8, the respective values were for controls (small: 11.8 ± 0.354%; large: 11.1 ± 0.424%) and infected/co-evolved (small: 11.7 ± 0.336%; large: 11.2 ± 0.357%) (Fig. 1). In generation 4, neither factor "population size" nor factor "coevolution with parasites" showed any significant effect on recombination rate (Table 1). In generation 8, parasites had again no effect, but large populations had a significantly reduced recombination rate compared to small populations ($P = 0.013$, Table 1). In the repeated ANOVA-analysis including generation 4 and 8, beetle line (p = 0.007, Table 2) and the interaction beetle-line x parasites ($P = 0.012$, Table 2) were significant indicating that different host lines responded differently to co-evolution by parasites.

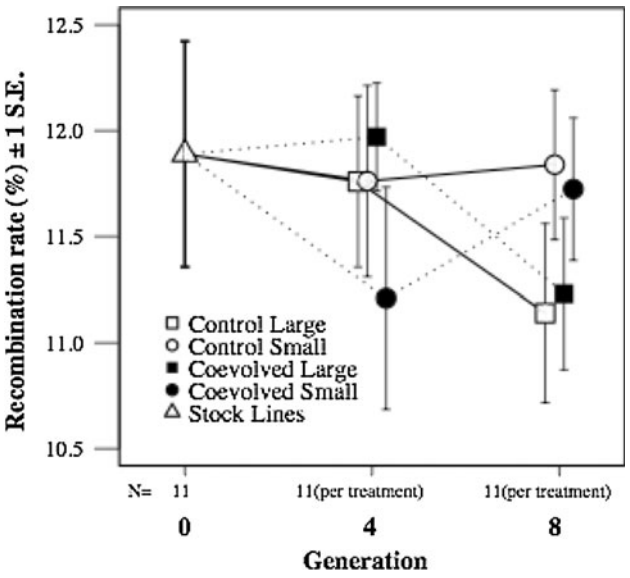

Fig. 1 Recombination rate (average of 4 genomic intervals) in either co-evolved or control, and either large or small populations. Females from large populations had a significantly lower recombination rate in generation 8 (ANOVA, $P = 0.013$) than those descending from small populations. *N* is number of replicate line (Sample size); further statistics in Tables 1 and 2

Table 1 General linear model for recombination rate for each generation separately

Recombination rate				
Source†	d.f.	MS	*F*	*p*
Generation 4				
Intercept	1	0.6	$1.91*10^{3}$	< 0.001
Error	10	$3.15*10^{-4}$		
Size	1	$1.58*10^{-4}$	0.912	0.362
Error	10	$1.73*10^{-4}$		
Parasites	1	$3.22*10^{-5}$	0.151	0.706
Error	10	$2.14*10^{-4}$		
Line	10	$3.15*10^{-4}$	1.01	0.485
Error	11.8	$3.11*10^{-4}$		
Size*Parasites	1	$1.6*10^{-4}$	2.1	0.178
Error	10	$7.62*10^{-5}$		
Size*Line	10	$1.73*10^{-4}$	2.27	0.106
Error	10	$7.62*10^{-5}$		
Parasites*Line	10	$2.14*10^{-4}$	2.8	0.06
Error	10	$7.62*10^{-5}$		
Generation 8				
Intercept	1	0.58	$2.91*10^{3}$	< 0.001
Error	10	$2*10^{-4}$		
Size	1	$3.91*10^{-4}$	8.99	0.013
Error	10	$4.35*10^{-5}$		
Parasites	1	$1.6*10^{-7}$	0.001	0.981
Error	10	$2.6*10^{-4}$		
Line	10	$2*10^{-4}$	0.959	0.551
Error	11.8	$2.08*10^{-4}$		
Size*Parasites	1	$1.16*10^{-5}$	0.122	0.734
Error	10	$9.51*10^{-5}$		
Size*Line	10	$4.35*10^{-5}$	0.457	0.883
Error	10	$9.51*10^{-5}$		
Parasites*Line	10	$2.6*10^{-4}$	2.73	0.064
Error	10	$9.51*10^{-5}$		

Factor "Size" is population size (large, small); "Parasites" is control or infected/co-evolving; "Line" is identity of beetle line

† Size, Parasites as fixed effects; Line as random effect

Fitness was measured in generation 4 and 8 as fecundity of the test females. In generation 4, estimates, were obtained for small (96.2 ± 7.31 offspring/female) and large control populations (91.0 ± 6.10), and for small (104 ± 9.17) and large infected/co-evolved populations (109 ± 9.74). In generation 8, these values were for controls (small: 99.3 ± 8.96; large: 98.7 ± 9.07) and infected/co-evolution (small: 99.1 ± 9.27; large: 102 ± 10.8) (Fig. 2). Co-evolved populations thus had significantly more offspring than control populations in generation 4

($P = 0.045$, Table 3). In generation 8, this effect could not be seen anymore. Note that this test was run under control (non-infected) conditions. Factor population size never showed any significant influence on fecundity. Beetle line per se significantly affected fitness in generation 4 ($P = 0.032$) and in generation 8 ($P < 0.001$). In generation 8, the interaction beetle line × population size was significant for fecundity ($P = 0.013$). Since females were allowed to lay eggs for 13 days in generation 4, but only for 10 days in generation 8, it is difficult to compare the results of the two generations.

Table 2 Repeated general linear model for recombination rate. The analysis was performed with the values of generation 4 and 8. Other factors like Table 1

Recombination rate				
Source[†]	d.f.	*MS*	*F*	*p*
Generation	1	$8.11*10^{-5}$	0.786	0.396
Generation*Size	1	$5.23*10^{-4}$	5.07	0.048
Generation*Parasites	1	$1.39*10^{-5}$	0.135	0.721
Generation*Line	10	$1.47*10^{-4}$	1.42	0.295
Generation*Size*Parasites	1	$4.28*10^{-5}$	0.415	0.534
Generation*Size*Line	10	$1.71*10^{-4}$	1.66	0.219
Generation*Line*Parasites	10	$1.61*10^{-4}$	1.56	0.249
Error	10	$1.03*10^{-4}$		
Intercept	1	1.18	$17.3*10^{3}$	< 0.001
Size	1	$2.59*10^{-5}$	0.38	0.551
Parasites	1	$1.84*10^{-5}$	0.271	0.614
Line	10	$3.68*10^{-4}$	5.4	0.007
Size*Parasites	1	$1.29*10^{-4}$	1.89	0.199
Size*Line	10	$4.56*10^{-5}$	0.67	0.731
Line*Parasites	10	$3.13*10^{-4}$	4.6	0.012
Error	10	$6.81*10^{-5}$		

[†] Generation as within subject factor;size, parasites and line as between subject factors

In order to test for trade-offs between recombination rates and fitness the two measurements were related to one another in a partial correlation correcting for beetle line, population size and parasites. Neither in generation 4 nor in generation 8, a significant correlation could be found (generation 4: $N = 40$, $r = 0.100$, $P = 0.558$; generation 8: $N = 44$, $r = -0.233$, $P = 0.143$). Hence, there was no relationship of recombination rate to fecundity in a given host line.

Interference

For all genomic intervals, changes in recombination rate between subsequent generations were calculated and compared to the corresponding changes in the adjacent interval on the same linkage group/chromosome (only 2 intervals were measured per linkage group). On linkage group nr.2, no significant correlation could be detected at any time (Pearson correlation. Generation 0–4: $N = 44$, $r = 0.129$, $P = 0.406$; Generation 4–8: $N = 44$, $r = 0.181$, $P = 0.239$; Generation 0–8: N = 44, r = 0.165, $P = 0.284$). Interestingly, the two intervals on linkage group nr.1 strongly and negatively correlated between generation 4 and 8 ($P = 0.003$, Fig. 3), and between generation 0 and 8 ($P = 0.017$, Fig. 3); between generation 0

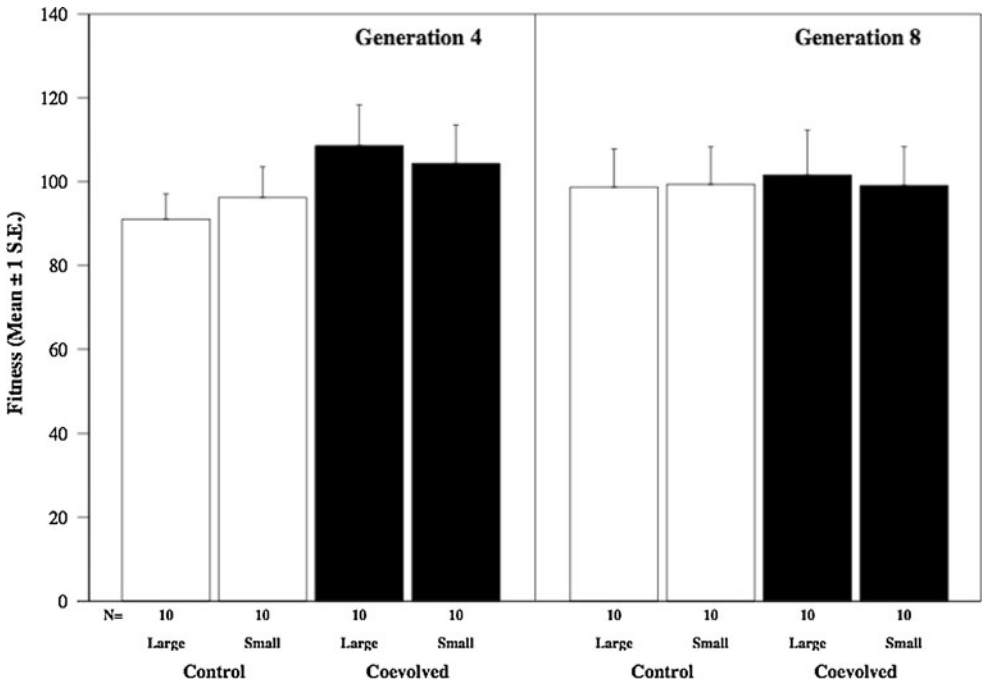

Fig. 2 Fitness measured as the number of beetles laid in generation 4 (during 13 days) and in generation 8 (during 10 days) on parasite-free flour. In generation 4, females from co-evolved populations had significantly more offspring than females descending from control populations (ANOVA, $P = 0.045$). Further statistical details in Table 3

Table 3 General linear model for fitness for each generation separately. Factors as in Table 1

Fitness				
Source†	d.f.	MS	*F*	*p*
Generation 4				
Intercept	1	$400*10^3$	212	< 0.001
Error	9	$1.89*10^3$		
Size	1	2.42	0.008	0.93
Error	9	298		
Parasites	1	$1.65*10^3$	5.43	0.045
Error	9	305		
Line	9	$1.89*10^3$	4.74	0.032
Error	6.38	398		
Size*Parasites	1	223	1.09	0.324
Error	9	205		
Size*Line	9	298	1.46	0.292
Error	9	205		
Parasites*Line	9	305	1.49	0.281
Error	9	205		
Generation 8				
Intercept	1	$437*10^3$	119	< 0.001
Error	10	$3.66*10^3$		
Size	1	8.94	0.048	0.831
Error	10	186		
Parasites	1	18.5	0.161	0.697
Error	10	115		
Line	10	$3.66*10^3$	14.1	< 0.001
Error	13.6	260		
Size*Parasites	1	27.6	0.671	0.432
Error	10	41.1		
Size*Line	10	186	4.53	0.013
Error	10	41.1		
Parasites*Line	10	115	2.79	0.061
Error	10	41.1		

† Size, Parasites as fixed effects; Line as random effect

and 4 no significant correlation was found on linkage group nr.1 ($P = 0.559$, Fig. 3).

Testing for plastic recombination

As explained in the Methods, we measured recombination rate based on 50 offspring of each female. The test was paired, since the respective mothers were either infected or non-infected but, at the same time, sisters of the same mother from a given beetle line. Offspring was raised parasite-free; a total of 10 sister-pairs were tested. The median recombination rate in control females was 11.8% (inter-quartile range 8.95–13.6%) and for infected females 11.0% (inter-quartile range 10.0–13.3%). A paired t-test revealed no significant difference in the average recombination rates ($t_9 = 0.326$, $P = 0.752$). Given the power of the test, we would have been able to detect a difference of 3.6 cM; the sample size needed to reach a generally accepted power of $(1\text{-}\beta) = 0.8$ with our observed, small difference is, however, unrealistically large ($n = 1{,}479$ pairs of females). Hence, we take this evidence as tentatively suggesting a lack of plastic recombination rather than as a final proof.

Discussion

Our experiment was set up such that each of the 11 replicate lines entered the experiment with a different background of linkage disequilibrium as suggested by (Otto and Barton 2001). Remarkably, recombination rate in these stock lines (Fig. 1) corresponded well with the average value of 12.0% found in the earlier experiment by (Fischer and Schmid-Hempel 2005) in their stock lines of *Tribolium castaneum* even though the beetle lines used in our experiment were not the same. This is also remarkable, since recombination rates appear to respond surprisingly quickly to selection. For example, populations of *T. castaneum* almost doubled rates after 15 generations of explicit selection for this trait (Dewees 1975). When high and low lines were crossed and back-crossed in that experiment, recombination showed unimodal frequency distributions which suggests multi-genic control of recombination rate and no major gene or chromosomal effects. By contrast, control by few major loci would have led to bimodal or multi-modal distributions, similar to any other phenotype under genetic control.

In our experiment, selection on altered recombination rates was indirect via the selection by added parasites. Fast changes as found in the study of (Dewees 1975) are therefore far beyond of what may be expected in our case. Nevertheless, in the earlier experiment where *T. castaneum* was experimentally co-evolved with the natural parasite *N. whitei* (microsporidia) (Fischer and Schmid-Hempel 2005) recombination rates at exactly the same intervals as in our study increased within the first eight generations by more than 10% in co-evolved compared to control populations (1–2 cM in absolute numbers). In our study, we could not detect the same effects; recombination was not different for infected and uninfected beetle populations. There are in fact a number of differences between the designs of the two studies. For example, the earlier study used an intermediate population size of 180 beetles. Moreover, all stock lines at the start of the experiment were pooled to form one large starting population from where the experimental replicate lines were derived. This mixing of stock lines might have broken up previously established (and therefore fit) allelic combinations. Recombination therefore might have been favoured more strongly by its effects on restoring fit allelic

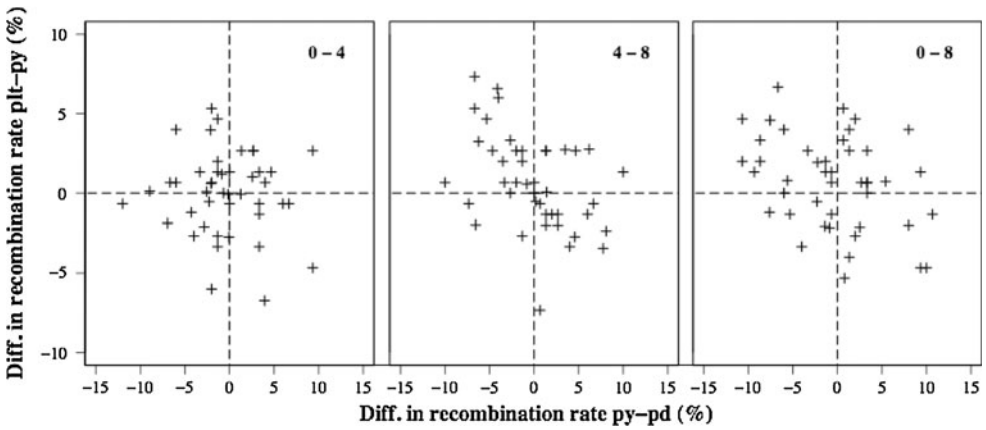

Fig. 3 Crossover interference on linkage group nr.1. Shown are the changes over time at one site in relation to changes over the same time at another site on the chromosome. No relationship was detected for changes in recombination rates between generation 4 and 8 (Pearson correlation, $r = -0.091$, $P = 0.559$, $N = 44$). Between generation 4 and 8 changes in recombination rates correlated negatively with each other ($r = -0.436$, $P = 0.003$, $N = 44$) as did changes in recombination rate between generation 0 and 8 (Pearson correlation, $r = -0.36$, $P = 0.017$, $N = 44$)

combinations. This effect should, however, be different for co-evolved and for control populations, and can thus not fully explain our results.

We here find a strong effect of population size, with increased recombination rates in small populations, as would be expected the Hill-Robertson model, where increased genetic drift combined with selection maintains negative LD, which is favourable for the spread of recombination (Otto and Barton 2001). However, if selection by parasites is strong (as is indeed the case for the highly virulent microsporidian *N.whitei)* we should also have observed a population size × treatment-interaction, which is not the case. Note, however, that the combined effects of drift and directional selection on recombination rate have been analysed theoretically, but little is known about the combined effects with fluctuating epistatic selection, which is more relevant for antagonistic host-parasite co-evolution studied here In computer simulations at least (Salathé et al. 2008), genetic drift caused by limited population size is necessary to keep linkage disequilibrium continuously fluctuating rather than converging to zero in infinite populations.

As an unexpected result, our experiment provides evidence for crossover interference between adjacent genomic intervals. At least for some comparisons, an increase over subsequent generations in recombination rate in one interval was related negatively to the change in recombination rate in the neighbouring interval (Fig. 3). This may indicate that recombination rate is adjusted locally or even in dependence on the rates of the surrounding parts of the genome. The phenomenon of interference is not entirely new though. In *D. melanogaster*, positive crossover interference – i.e. one crossover reduces the probability of another crossover in an adjacent site (Zhao et al. 1995)—was shown already in the 1930's (Weinstein 1936). Interference operates in most eukaryotes assayed to date, acting over whole chromosomes or chromosome arms (Hillers 2004; Van Veen and Hawley 2003). The biological reasons and mechanisms for this phenomenon are still speculative. Such local effects could corrupt our data in a way that no net changes are detected locally although the recombination rate is altered globally.

In the fourth generation, beetles derived from co-evolved populations had a significantly higher fitness than beetles derived from control populations. In our setup, fitness was measured in F1-beetles, which never encountered parasites directly (*N. whitei* is not transmitted transovarially (Milner 1972)). We have no ready explanation for this, except that co-evolution with parasites might have simultaneously selected for generally fitter beetles, for example, with respect to metabolic efficiency in the face of costly immune defences (Moret and Schmid-Hempel 2000). Often, increased parasite resistance is thought to correlate negatively with other fitness components (Hasu et al. 2006; Schmid-Hempel 2003). Our data could not corroborate this expectation, as there was no correlation between recombination rate and fecundity across the tested females. Finally, we could not detect any hard evidence for an infection-induced change in recombination rate even though this finding can only be suggestive at the time being. Such plasticity has been reported for various other systems. For example, starvation induced higher recombination rates in yeast, *Saccharomyces cerevisiae* (Meyen), (Abdullah and Borts 2001) and *D. melanogaster* (Neel 1941). Similarly, breeding either below or above the optimal temperature led to an increase in recombination rate in earlier (Plough 1917; Plough 1921) and more recent studies of *D. melanogaster* (Grell 1978). Other abiotic stress factors such as ionizing radiation, mitomycin C, increased

salinity and heat are also known to stimulate somatic recombination in plants (Lebel et al. 1993; Puchta et al. 1995). In *Arabidopsis thaliana* (L.) Heynh. In tobacco, *Nicotiana tabacum*, infection systemically increases recombination rates in leaf tissues were reported recently (Lucht et al. 2002) (Kovalchuk et al. 2003).

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