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Biol. Lett. 2005 **1**, 193-195
doi: 10.1098/rsbl.2005.0296

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biology letters

Biol. Lett. (2005) **1**, 193–195
doi:10.1098/rsbl.2005.0296
Published online 24 May 2005

Selection by parasites may increase host recombination frequency

O. Fischer and P. Schmid-Hempel*

ETH Zürich, Ecology & Evolution, ETH-Zentrum NW, CH-8092 Zürich, Switzerland
**Author for correspondence (psh@env.ethz.ch)*

Meiotic recombination destroys successful genotypes and it is therefore thought to evolve only under a very limited set of conditions. Here, we experimentally show that recombination rates across two linkage groups of the host, the red flour beetle *Tribolium castaneum*, increase with exposure to the microsporidian parasite, *Nosema whitei*, particularly when parasites were allowed to coevolve with their hosts. Selection by randomly varied parasites resulted in smaller effects, while directional selection for insecticide resistance initially reduced recombination slightly. These results, at least tentatively, suggest that short-term benefits of recombination—and thus the evolution of sex—may be related to parasitism.

Keywords: recombination; sex; evolution; parasites; selection

1. INTRODUCTION

Recombination can only evolve under a very limited set of conditions (Barton 1995; Otto & Michalakis 1998). Advantages result when favourable gene combinations are under-represented (linkage disequilibrium with respect to epistasis) owing to, for example, genetic drift in small populations (Otto & Barton 2001). Alternatively, antagonistic host–parasite coevolution, perhaps in combination with other factors (Otto & Nuismer 2004), can cause rapidly fluctuating epistatic selection that generates favourable conditions during short but repeated episodes (Hamilton *et al.* 1981) predicted to last for three to five generations (Barton 1995; Peters & Lively 1999). Antagonistic coevolution by parasites selects for novel recombinant host types that can escape the rapidly adapting parasite population of previous generations (Hamilton *et al.* 1981). The corresponding advantage of recombination should be lower when every new host generation are infected by a random set of (non-coevolved) parasites. In addition, increased recombination can also evolve in response to directional selection (e.g. caused by an insecticide), owing to increased additive genetic variation after the breakdown of linkage disequilibrium (Barton 1995). Here, we have tested how different selection regimes affect the evolution of recombination frequency in the red flour beetle, *Tribolium castaneum*.

2. MATERIAL AND METHODS

We have exposed populations of *T. castaneum* to selection by the natural parasite, *Nosema whitei*. *Nosema whitei* infects the larvae and occasionally persists into the adult, causing increased mortality rates and reduced fecundity (West 1960; Armstrong & Bass 1986). Infection occurs by ingestion of spores contained in the flour. Stocks of *T. castaneum* were bred from original material obtained from the USDA Grain Marketing Research Laboratory. Different populations were maintained separately for 10–15 generations before the start of the experiment. For each treatment, we used 10 replicate lines created by random assignment of beetles from the start-up populations. Lines were maintained at 180 beetles each (30 beetles until second generation) in 1.3 l jars (80 g of sterilized flour, 5% yeast, at 33 °C, 80% relative humidity; generation time approximately 28 days). To form discrete generations, beetles were allowed to lay eggs into the medium before being removed after 7 days; 28–38 days after egg laying, 180 newly hatched beetles of the next generation were sampled and transferred into a new jar.

In the coevolution treatment, the next generation of beetles was exposed to *N. whitei* spores taken from the previous host generation of the same experimental line. In the random treatment, the next generation of beetles was exposed to a random sample of spores extracted from a total of 12 external beetle lines kept infected independently of the experiment. For infections we used a standard dose of 10^3 spores per gram of medium. In the insecticide treatment, the beetles were exposed (for 24 h) to malathion (1.5 mg in 0.5 ml xylol) applied on a filter paper (Ahuja 1985). Then, 180 of the survivors were transferred to standard medium as breeders of the next generation. Control lines were handled in identical ways but not exposed to insecticide nor parasites.

Generations 0, 4, 8 and 12 were surveyed for recombination frequency. For this, we used two beetle strains homozygous for recessive markers with unambiguous expression on linkage group I (markers plt, py, pd) and linkage group II (ub, pas, apt), respectively. Because the location of markers physically is fixed in the genome (Sokoloff *et al.* 1987), the map lengths (usually measured as recombination distance in centimorgans (cM)) for the respective genetic intervals between markers differ irrespective of treatment (plt–py, 18.0 cM; py–pd, 9.0 cM; ub–pas, 16.0 cM; pas–apt, 4.7 cM). This intrinsic difference was taken into account in the statistical analysis with the fixed factor 'interval'. Experimental selection changes the average value of the recombination distance of these intervals.

To measure recombination frequencies, five to six virgin females (for each (of 10) experimental line, each (of 4) treatment, and for generations 4, 8 and 12) were crossed with males from the marker strains and offspring raised under control (non-infected) conditions. These F1 offspring were then backcrossed to marker males. The resulting F2 offspring were again raised under control conditions and scored for the number of parental or recombinant types. This scheme estimates recombination in the F1 females and exposes differences inherited from their parents (which had been subject to treatment). Pre-selection frequencies (for generation 0) were estimated from a sample of 20 randomly chosen females from the start-up population. A total of 100–150 offspring for each original female (per generation and line) and linkage group were scored, i.e. a total of 1000–1500 beetles per treatment, generation and linkage group; over 30 000 beetles in the experiment.

We used ANOVA with factors 'treatment' and 'interval' entered as (between-subject; fixed) factors, and line as a nested (random) factor within treatment. This analysis was applied to the data in every measured generation. Except for the effect of directional selection by the insecticide, *a priori* it is unclear whether the effect of fluctuating selection by parasites on recombination after any number of generations will always accumulate. Therefore, we considered the estimates obtained on the same selection line in generations 4, 8 and 12 as repeated measures (within-subject factor; repeated-measures ANOVA) for the cumulative effect of treatment. This model also yielded estimated means for the overall recombination rate after all of the above factors have been taken into account (cf. figure 2). All analyses were run with SPSS 11 for Mac OS X.

3. RESULTS

The parasite treatments showed a different pattern as compared with selection by insecticide (see table 2 and figure 1). For generations 4 and 8 as well as for the overall experiment, there was a significant overall treatment effect (figure 2 and tables 1 and 2).

Received 29 November 2004
Accepted 10 January 2005

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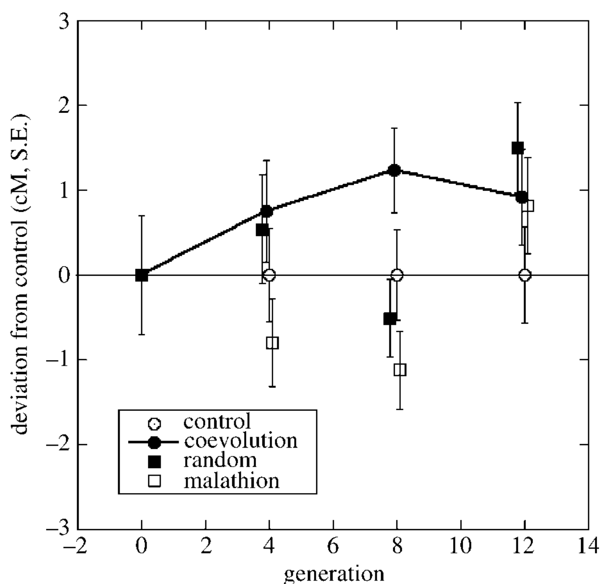

Figure 1. Observed recombination frequencies in cM. Shown are the deviations from control (horizontal line) associated with treatment (solid line, coevolution), and after the factorial effects of interval and line have been taken into account (i.e. estimated marginal means from the statistical model). Symbols have been offset slightly for clarity. For statistics, see tables 1 and 2.

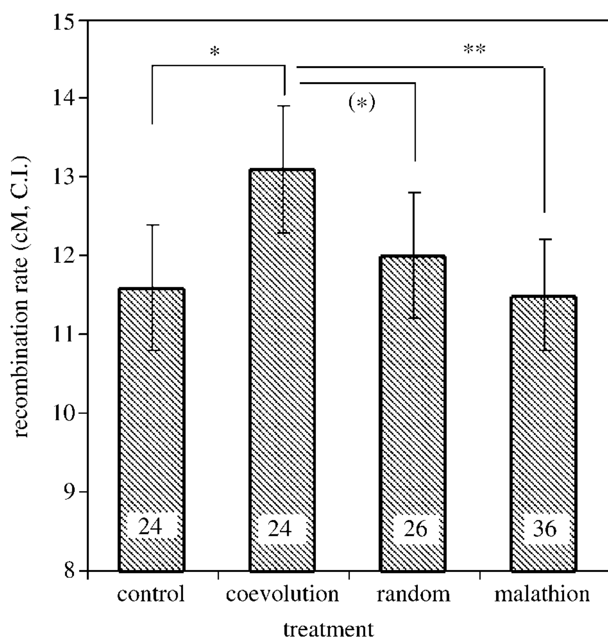

Figure 2. Recombination (in cM) varied with treatment (shown are estimated marginal means from the statistical model; internal numbers are sample sizes). Within the ANOVA, coevolution differed from control (difference contrast, $p=0.013$) but not from random ($p=0.50$) while random was marginally different from malathion ($p=0.09$). In independent pairwise comparisons, coevolution was different from control ($p=0.013$), malathion ($p=0.005$) and, marginally, from random ($p=0.071$); respective significance levels are indicated by asterisks (**$p\leq 0.01$, *$p\leq 0.01$, (*) one-tailed $p\leq 0.05$).

As expected, recombination intrinsically differed among intervals (owing to differences in physical map length) while line had no effect nor did treatment effects differ among intervals (see interaction terms, tables 1 and 2). Comparing treatments for the overall experiment, coevolution differed from control and malathion but only marginally from random, while random was marginally different from malathion (figure 2). Coevolution deviated from other treatments especially in generation 8 (table 2).

Table 1. ANOVA for the overall experiment (see text). (Significant effects in bold.)

source[a]	d.f.	*F*	*p*
treat	3	3.253	**0.028**
interval	3	298.989	$<$**0.001**
line (treat)	32	0.616	0.931
interval*treat	9	0.943	0.495
error	62		

[a] Treat, interval as fixed effects. Line as random effect within treat.

Table 2. ANOVA for each generation (see text). (Significant effects in bold.)

source[a]	d.f.	*F*	*p*
generation 4			
treat	3	3.105	**0.034**
interval	3	152.393	$<$**0.001**
line (treat)	34	0.435	0.996
interval*treat	9	0.685	0.720
error	82		
generation 8			
treat	3	4.325	**0.010**
interval	3	217.038	$<$**0.001**
line (treat)	36	0.985	0.506
interval*treat	9	1.669	0.109
error	88		
generation 12[b]			
treat	3	1.502	0.229
interval	3	133.295	$<$**0.001**
line (treat)	36	0.837	0.724
interval*treat	9	1.544	0.143
error	102		

[a] Treat, interval as fixed effects. Line as random effect within treat.
[b] Comparing pooled treatments with control: $F_{1,128}=2.864$, $p=0.093$.

4. DISCUSSION

We did not directly select for recombination itself but compared selection regimes, which are hypothesized to provide benefits to recombination. In fact, antagonistically coevolving parasites continuously create novel environments for their host (Hamilton *et al.* 1981; Peters & Lively 1999). To some extent, this should also be the case for the random treatment, which may explain why there was no large difference to coevolution. In generation 12 all treatments converged (table 1), for which we have no ready explanation. On the other hand, the predicted long-term sustained rates of recombination under antagonistic coevolution are actually not very high and (at least in theory) may sometimes revert to lower levels after a rapid increase (Schmid-Hempel & Jokela 2002).

In our experiment, the rapid change over a few generations is the most striking result even though it agrees with the predicted short-term benefits for

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recombination (Peters & Lively 1999; figure 1). Numerous studies in *T. castaneum* show that experimental selection for almost any trait occurs within a few generations (e.g. Soliman 1982; Cardin & Minvielle 1986) while mutation can rapidly add substantial genetic variance (Enfield & Braskerud 1989). These studies are based on fewer than our 10 replicate lines, making it unlikely that our results only reflect random fluctuation (genetic drift). Studies also show that two main characteristics necessary for the evolution of recombination—linkage disequilibria and epistasis—are affecting various traits in *T. castaneum* and can rapidly change as a result of selection under similar conditions to those we have used (Carbonell *et al.* 1985; Silvela *et al.* 1999). Recombination can be changed by experimental selection, sometimes even within a few generations (Harinarayana & Murty 1970). Similarly, sexual variants (i.e. with recombination) quickly outcompete their asexual counterparts in novel environments (Rice 2002). Recently, it was found that pathogen infection increases recombination rates in somatic tissues of plants and generates a systemic signal (Kovalchuk *et al.* 2003). However, such plasticity could not explain our results either, since we always measured recombination rates in uninfected animals.

Little is known about the genes responsible for the modification of recombination rate in *T. castaneum* or the genes affecting resistance to *N. whitei*. Although the genetic locus affecting malathion resistance is located in linkage group VI (Beeman 1983). Recently, quantitative trait loci for susceptibility to tapeworm infections were identified in several linkage groups (Zhong *et al.* 2003), with digenic epistatic effects explaining a major part of the variation.

This is the first study directly and experimentally testing the effect of host–parasite coevolution on recombination rates. Because we could only measure recombination rates for two linkage groups, the genome-wide effects may not be reflected. Furthermore, we bred the population for 10–15 generations to remove any disequilibrium present in the stock population, but many more generations would be required to eliminate all disequilibrium, especially between closely linked loci. Thus, it remains theoretically possible that our results could be explained by the presence of initial disequilibrium in the stock population, between a modifier of recombination and a locus subject to selection by parasites. Further experiments using different stock populations, with presumably different genetic associations among loci, will be needed to assess this possibility. Nevertheless, the current results are encouraging and can guide further tests to establish the proper role of antagonistic host–parasite interaction for the evolution of recombination.

We acknowledge the stocks provided by R. W. Beeman (USDA) and D. Ebert (Unversity of Fribourg). We thank P. Korner, L. Pétro and J. Vogel for help. S. Bonhoeffer, R. Schmid-Hempel and J. Jokela provided critical comments and statistical advice.

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