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Report

A Matching-Allele Model Explains Host Resistance to Parasites

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Summary

The maintenance of genetic variation [1–3] and sex [4–8] despite its costs [9] has long puzzled biologists. A popular idea, the Red Queen Theory [4–8], is that under rapid antagonistic coevolution between hosts and their parasites, the formation of new rare host genotypes through sex can be advantageous as it creates host genotypes to which the prevailing parasite is not adapted. For host-parasite coevolution to lead to an ongoing advantage for rare genotypes, parasites should infect specific host genotypes and hosts should resist specific parasite genotypes. The most prominent genetics capturing such specificity are matching-allele models (MAMs), which have the key feature that resistance for two parasite genotypes can reverse by switching one allele at one host locus. Despite the lack of empirical support, MAMs have played a central role in the theoretical development of antagonistic coevolution [4, 7, 8, 10], local adaptation [11, 12], speciation [13], and sexual selection [14]. Using genetic crosses, we show that resistance of the crustacean *Daphnia magna* against the parasitic bacterium *Pasteuria ramosa* follows a MAM. Simulation results show that the observed genetics can explain the maintenance of genetic variation and contribute to the maintenance of sex in the facultatively sexual host as predicted by the Red Queen Theory.

Results and Discussion

The planktonic crustacean *Daphnia magna* is a cyclic parthenogen, allowing for genetic crosses to be performed and the resulting recombinants to be maintained as clonal lineages. Two *Daphnia* genotypes (Fa and X) from a rock pool metapopulation in southwestern Finland were selfed for three rounds and then used to create a set of crosses (Figure 1). Multiple replicates of each recombinant genotype were tested for resistance against two genotypes of *P. ramosa* (C1 and C19) via two methods, attachment and infection trials [15, 16]. These *P. ramosa* genotypes differ in the *Daphnia* genotypes they can infect and were known to differentially infect the parents of our crosses. Recombinant offspring of all crosses were fertile and no differences in hatching probabilities from resting eggs were detected. We observed Mendelian segregation of resistance for both parasites in two separate crosses (F2 and parent 1 selfed) (Table 1 and Figure 1A). In both cases, resistance was dominant and susceptibility was recessive. A backcross (F1 with parent 1) revealed that individuals were either susceptible to both *P. ramosa* clones (26%) or were only resistant to one (C19; 23%) or the other (C1; 51%). The absence of individuals resistant to both *P. ramosa* genotypes indicated that resistance to these parasites was not independently inherited. Indeed, selfing of ten of the backcross genotypes showed that those observed to be C1 resistant were in fact comprised of two different genotypes (Figure 1B). These results may either reflect the presence of intralocus interactions (dominance) or indicate interlocus interactions (epistasis). Under the dominance model, resistance would be coded for by a single locus with three alleles (x, y, z) with a dominance hierarchy. Allele x provides resistance against C1 but susceptibility to C19, which is dominant over allele y, which provides resistance against C19 and susceptibility to C1. In addition, both resistance alleles (x and y) show dominance over a third allele, which does not confer resistance to the either *P. ramosa* C1 or C19. Under the epistasis model, resistance would be coded for by two loci, each with two alleles (A/a and B/b), dominant resistance (C1 resistance allele "A" and C19 resistance allele "B"), and epistasis between both dominant resistance alleles. Individuals carrying the "A" allele are susceptible to *P. ramosa* C19 regardless of the presence of the "B" allele, which, in the absence of "A," normally confers resistance to C19. To distinguish between both types of interactions, we performed multiple crosses (n = 118) with two backcrossed genotypes (c*d, Figure 1B and Table 1). The absence of double susceptible individuals indicated that a dominance hierarchy on one locus could explain the data. Presence of epistasis between two loci may still explain the data, but only if the loci are closely linked. Recombination would be so rare that it is difficult to detect it in breeding studies and the two loci would behave like a single locus. Our findings are consistent with the results from a previous study that speculated that resistance of *D. magna* against two isolates of *P. ramosa* was dominant for one and recessive for the other isolate [17].

This study of host resistance genetics in the *Daphnia-Pasteuria* system provides the first empirical evidence consistent with a matching-allele model (MAM). Characteristic of MAMs is that a parasite can only infect when its alleles match those of its host or a host can only resist the parasite when its alleles matches that of the parasite. This key feature has been implemented in different ways in the family of MAMs, such as inverse matching versus matching [12, 18], variable number of matching loci [18, 19], variable number of alleles per locus [11, 18, 19], and different ploidy levels of hosts and parasites [5, 8, 10]. The important aspect distinguishing MAMs from alternative genetic models is that the resistance/susceptibly effects for the two parasites can reverse by switching an allele at one host locus. Both our dominance (one-locus) and epistasis (two-locus) models fulfill this criterion (under the two-locus model a substitution at a single locus can switch the outcome of the infection process due to the epistasis between both loci). Furthermore, under both the dominance and

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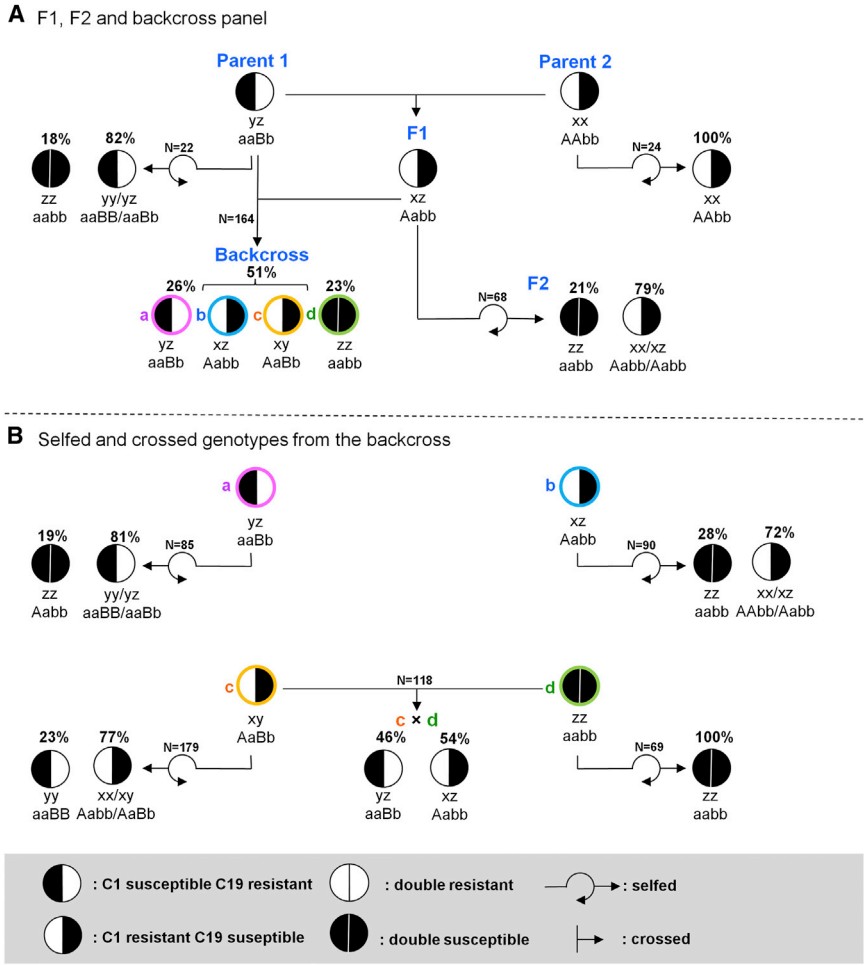

Figure 1. Pedigree Showing the Crossing Scheme and Resistance Profiles of *Daphnia magna* Genotypes against Two *Pasteuria ramosa* Genotypes, C1 and C19

The F1, F2, and backcross (A) and selfing of, and crosses among, ten backcrossed individuals (B) are shown. "N" represents the total number of recombinants tested over multiple independent crosses (details in Table 1) and percentages reflect segregation patterns. Two genetic models are shown: a one-locus model with alleles z, y, and x and a two-locus model with two alleles at each locus (A,a and B,b).

epistasis models, resistance/susceptibility only occurs in specific combinations of host and parasite alleles (Table 2). Although we only used a small subset of host and parasite genotypes, the here-found genetics may apply to other combinations of *P. ramosa* and *D. magna* as high specificity, a signature from MAMs, has been found for numerous *P. ramosa-D. magna* combinations [20–22]. Although (inverse) gene-for-gene models [23, 24] can also result in such specificity these models also predict the presence of universally resistant hosts or universally infective parasites and previous studies suggest that these do not occur in the *Daphnia-Pasteuria* system [e.g., 20–22]. These studies tested diverse hosts and parasite isolates and did not find any host clone that was resistant to all parasites or any parasite isolate that was infective in all host clones. Furthermore, time series using archived *D. magna* and *P. ramosa* resting stages from lake sediment cores did not show any selective sweeps associated with universally infective parasites or universally resistant host genotypes [25]. Irrespective of the underlying genetics of resistance for untested combinations of *P. ramosa* and *D. magna* the here-found reversal in resistance by switching a single allele at one host locus confirms the key feature of MAMs. This criterion can be applied even if only part of the interaction matrix is known.

Under a matching-allele model, infection is based on a recognition process between host and parasites. In the *Daphnia-Pasteuria* system, recognition occurs when ingested spores attach to the esophagus of susceptible hosts. Upon successful attachment, *P. ramosa* enters the host and proliferates in the hemolymph and muscle [15]. A putative explanation consistent with our results and others [15] is that gene products of the loci/locus described here prevent attachment of *P. ramosa* to the host esophagus either by actively disrupting adherence of *P. ramosa* spores or by blocking target receptors. Such a mechanism would fit with the family of matching-allele models, which are based on the ability of the host to recognize and resist attack by parasites [11, 18].

MAMs have played a central role in the theoretical development of antagonistic coevolution and were shown to readily lead to time lagged negative frequency dependent selection, maintenance of genetic variation [1, 10, 18] and sex [4, 5, 8], pertinent issues in ecology and evolutionary biology. A simulation model (for model methods, see the Supplemental Experimental Procedures available online) based on the here-observed genetics indeed shows that coevolution between *Daphnia* and *Pasteuria* can maintain genetic variation indefinitely (Figure 2). Empirical studies are consistent this observation: the *Daphnia-Pasteuria* system is one of the few systems with evidence for rapid antagonistic coevolution, consistent with negative frequency dependent selection [25, see 26, 27 for other systems]. Furthermore, the observation of high within-population variation for resistance [20] but low among-population variance [28] is consistent with expectations for traits experiencing such a selection regime [29]. The *Daphnia* system can now be used to empirically test specific predictions of the Red Queen Theory, such as negative frequency-dependent selection and the notion that antagonistic coevolution may favor sex. Models show that sex is only favorable under a limited set of conditions [5, 8], and it remains an open question whether real biological systems fall within this parameter space. Our simulation model suggest that the *D. magna-P. ramosa* system may fall into this parameter space as simulations with realistic parameter settings show an advantage for sex by segregation that may partly alleviate or overcome the costs of sex (Table S2). Although the majority of studies attempting to explain the widespread occurrence of sexual reproduction by antagonistic coevolution have focused on the effects of recombination

Table 1. Details of Each Cross of the Breeding Scheme Shown in Figure 1

Cross (as Shown in Figure 1)	Expected Genotype under the One-Locus Model	Expected Genotype under the Two-Locus Model	Observed/Expected (%) C1 sus, C19 sus	C1 sus, C19 res	C1 res, C19 sus	C1 res, C19 res	n	p Value
Parent 1	yz	aaBb	0/–	100/–	0/–	0/–	1	–
Parent 2	xx	AAbb	0/–	0/–	100/–	0/–	1	–
Selfed parent 1	yz · yz	aaBb · aaBb	18/25	82/75	0/0	0/0	22	0.72
Selfed parent 2	xx · xx	Aabb · AAbb	0/0	0/0	100/100	0/0	24	1.00
F1	xx · yz	Aabb · aaBb	0/0	0/0	100/100	0/0	1	–
F2	xz · xz	Aabb · Aabb	21/25	0/0	79/75	0/0	68	0.69
Backcross	xz · yz	Aabb · aaBb	23/25	26/25	51/50	0/0	164	0.94
a clone 1 selfed	yz · yz	aabb · aabb	21/25	79/75	0/0	0/0	38	0.79
a clone 2 selfed	yz · yz	aabb · aabb	17/25	83/75	0/0	0/0	47	0.45
b clone 1 selfed	xz · xz	Aabb · Aabb	19/25	0/0	81/75	0/0	26	0.74
b clone 2 selfed	xz · xz	Aabb · Aabb	31/25	0/0	69/75	0/0	64	0.56
c clone 1 selfed	xy · xy	AaBb · AaBb	0/0	17/25	83/75	0/0	35	0.56
c clone 2 selfed	xy · xy	AaBb · AaBb	0/0	25/25	75/75	0/0	68	1.00
c clone 3 selfed	xy · xy	AaBb · AaBb	0/0	22/25	78/75	0/0	49	1.00
c clone 4 selfed	xy · xy	AaBb · AaBb	0/0	30/25	70/75	0/0	27	1.00
d clone 1 selfed	zz · zz	aaBb · aaBb	100/100	0/0	0/0	0/0	30	1.00
d clone 2 selfed	zz · zz	aaBb · aaBb	100/100	0/0	0/0	0/0	39	1.00
c × d crosses 1[a]	zz · xy	aabb · AaBb[b]	0/0	45/50	55/50	0/0	62	0.85
c × d crosses 2[a]	zz · xy	aabb · AaBb[b]	0/0	46/50	54/50	0/0	56	0.72

The table shows the total number of host clones tested per cross (n) and the percentage of these clones that where either susceptible (sus) or resistant (res). The top part of the table shows the parental, F1, F2, and backcross, and the bottom shows results obtained from selfing and crossing of ten genotypes from the backcross. The p value represents a Fisher's exact test between the number observed and expected under both genetic models.
[a]Crosses were done in two blocks.
[b]As expected under perfect linkage.

among loci [4, 5, 10, 19], theoretical work shows that in diploids segregation may overwhelm the effects of recombination and that segregation alone can under some conditions favor sex [8].

A MAM is the most extreme form of specificity in biological interactions and has been assumed in many theoretical models addressing questions not only related to the maintenance of sex and genetic diversity, but also to local adaptation [11, 12], speciation [13], and sexual selection [14]. Our results show for the first time, that different alleles at the same locus can indeed select for specific genotypes of an antagonist, confirming a key assumption of these models. Thus, our results close a long-standing gap between theory and real biological systems. Findings from theoretical models that use the matching-allele assumption therefore gain substantially credibility.

Table 2. The *Daphnia-Pasteuria* Genetics Matrix

Host Genotype under the One-Locus Model	Host Genotype under the Two-Locus Model	Parasite Genotype: C1	Parasite Genotype: C19
xx	AA−−	resistant	susceptible
xy/xz	Aa−−	resistant	susceptible
yy/yz	aaB−	susceptible	resistant
zz	aabb	susceptible	susceptible

The matrix follows a matching-allele model (see Table S1) because replacement of one allele "y" versus "x" or "a" versus "A" in the one- and two-locus models, respectively, reverses resistance to the two parasite genotypes. Double susceptible animals will likely be selected against and these alleles may be lost, unless they provide resistance against untested genotype of *P. ramosa* or if resistance alleles carry a cost. The "−" represents the presence of an allele that has no influence on the phenotype due to epistasis or dominance. See also Table S1 for a matching-allele model for a diploid host and haploid parasite.

Experimental Procedures

Details for crosses and both methods used to assess resistance (infection trials and attachment tests) are described in a previous study [16], in which assays with *P. ramosa* genotype C19 were similar to C1, protocols for the selfed backcross were identical to those of the selfed parents, and protocols for crosses between two pairs of the backcross were identical to those of the backcross. In short, selfed lineages were produced by hatching of sexual resting eggs from monoclonal laboratory populations of their respective parent clone. For the creation of the F1 offspring, we collected sexual resting eggs from a mixed laboratory population of both parents and used microsatellites to distinguish between selfed and outcrossed offspring. For the other crosses, we placed virgin females of one parent together with males of the other and removed all parthenogenetic offspring to prevent selfing. All sexual resting eggs were removed as they appeared, dried

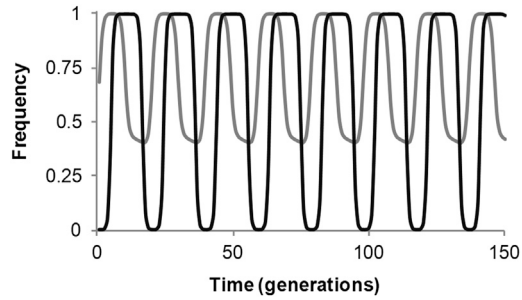

Figure 2. Maintenance of Genetic Variation through Negative Frequency-Dependent Selection

Under all parameter settings of the simulation model, genetic variation was maintained. The figure shows cycles of antagonistic coevolution between *Daphnia* and *Pasteuria* (parameter settings v = 0.8 and t = 0.9). Gray, frequency of the host allele for C19 resistance and C1 susceptibility; black, frequency of parasite C1. The figure shows the dynamics after a burn in of 10,000 generations. Simulation model methods can be found in the Supplemental Experimental Procedures. See also Table S2 for simulation model results on the evolution of sex.

for at least 14 days, hatched, and maintained as clonal lineages in the laboratory. Four individuals of each recombinant lineage were tested with the attachment test. This test visualizes the attachment of the spores to the host esophagus by using fluorescence-labeled spores and correlates perfectly with host susceptibility [15]. For a subset of the crosses, we also performed infection trials by exposing up to eight *D. magna* females per recombinant genotype individually to 200,000 spores of *P. ramosa* and scoring infection status 30 days after exposure. All statistics were performed with Fisher's exact test.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.04.064.

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