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WHERE ARE THE WORMY MICE? A REEXAMINATION OF HYBRID PARASITISM IN THE EUROPEAN HOUSE MOUSE HYBRID ZONE

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Wormy mice in a hybrid zone have been interpreted as evidence of low hybrid fitness, such that parasites contribute to species separation. However, because of its natural heterogeneity, observations of parasite load must be numerous with good field area coverage. We sampled 689 mice from 107 localities across the Bavaria-Bohemia region of the European house mouse hybrid zone and calculated their hybrid indices using 1401 diagnostic single nucleotide polymorphisms (SNPs). We tested whether hybrids have greater or lesser diversity and load of parasite helminths than additive expectations, performing load analyses on the four most common taxa. We found hybrids have significantly reduced diversity and load of each of the commonest helminths; rarer helminths further support reduced load. Although within-locality comparisons have little power, randomization tests show the repeated pattern is unlikely to be due to local parasite heterogeneity, and simulations show a patch of low parasite diversity is unlikely to fall by chance just so in the field area, such that it produces the observed effects. Our data therefore contradict the idea that helminths reduce hybrid fitness through increased load. We discuss a vicariant Red Queen model that implies immune genes tracking parasites will escape Dobzhansky–Muller incompatibilities, generating hybrid variants untargeted by parasites.

KEYS WORDS: Helminths, immune gene transitive compatibility, *Mus musculus domesticus*, *Mus musculus musculus*, resistance.

In nature, taxa that have diverged in isolation may hybridize on secondary contact, and often form relatively stable and narrow hybrid zones maintained by dispersal of each taxon into the zone, and selection against hybrids (Bazykin 1969; Barton and Hewitt 1985). The genotypic/genetic study of secondary contact hybrid zones has led to important advances in understanding the nature of species and speciation, the possibilities for exchange of inherited elements across taxon boundaries (Barton 1979; Rieseberg et al. 1999), and the selective forces acting. Among these forces, parasitism has the potential to affect the outcome of hybridization

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by differentially impacting the fitness of host lineages and their hybrid descendants (Coustau et al. 1991; Fritz et al. 1999; Wolinska et al. 2006) although such an impact has never been clearly demonstrated in nature.

In Europe, two subspecies of the house mouse, *Mus musculus musculus* and *M. m. domesticus*, having diverged for some hundreds of thousands of generations in isolation (Boursot et al. 1993; Geraldes et al. 2008), and now differing in pelage color, aggression, relative tail length, and other traits (Berry 1981; Sage 1981; Brain et al. 1989), meet and hybridize. Although the house mouse hybrid zone (HMHZ) is more than 2500-km long, running from Norway and Denmark to the Black Sea, it is only about 20-km wide (Macholán et al. 2007; Jones et al. 2010). Variation of allozyme markers traced across the hybrid zone shows a great diversity of recombined genotypes in the center of the zone and introgressed individuals on both sides. There are neither F1s nor early-generation hybrids (Macholán et al. 2007). Spatial genetic analyses indicate this is a tension zone dominated by endogenous (genetic) factors rather than exogenous (environmental) factors (Barton and Hewitt 1985; Macholán et al. 2007, 2008). The sharper transition of sex chromosome markers versus autosomal markers (Tucker et al. 1992; Macholán et al. 2007) is consistent with endogenous selection against hybrids, as Dobzhansky–Muller incompatibilities (DMIs) are expected to be overrepresented on sex chromosomes (Orr 1997; Coyne and Orr 2004). This was one of the first animal hybrid zone models investigated with respect to the role of parasitism (Sage et al. 1986a).

Parasitological interest in this system was triggered by "wormy mice" found in hybrids where the HMHZ runs through southern Germany (Sage et al. 1986a). Of 93 individuals collected, 14 of 46 individuals classified as hybrids showed over 500 pinworms per gut, far exceeding the mean of 40 per gut of the individuals classified as parental. The authors suggested that a breakdown of coadapted gene complexes involved in parasite resistance would explain this pattern. Subsequently, Moulia et al. (1991) described a similar pattern for mice from the Danish section of the zone and demonstrated that pinworm susceptibility in mouse strains bred from wild-caught mice had a genetic component (Moulia et al. 1993). Although the 1986 and 1991 studies reported similar patterns of parasite load across two HMHZ transects, the sample sizes (93 and 120 mice, respectively) appear low given the spatial and seasonal heterogeneities that are characteristic of parasite occurrence and abundance (Kisielewska 1970; Haukisalmi et al. 1988; Montgomery and Montgomery 1989; Behnke et al. 1999). There are also limitations in the sampling design of both studies. The 1986 dataset considers just 22 localities spanning 200 km, some of which were sampled in autumn and others in spring of the following year (Sage et al. 1986a). Seasonal variation in parasite load could be a large component of interlocality variation in such a dataset. Regarding the 1991 dataset, 12 localities spanning ≈150 km (one on an offshore island), the field-caught mice were maintained for two months in laboratory conditions before being dissected for parasite counts (Moulia et al. 1991). Therefore, these counts may not reflect the parasite load the mice had when they were in the field. Subsequent to the 1986 and 1991 studies, experimental infections of individuals from the original taxa and crosses (from F1 to F4) were carried out with the pinworm *Aspiculuris tetraptera*. These failed to reproduce high worm loads in the hybrids, in fact showing reduced hybrid load (Derothe et al. 2004). Finally, it should be noted that the definition of a hybrid varies across these studies. A hybrid index (*HI*) can be used to place any mouse on a linear scale from *musculus* to *domesticus* depending on the count of *domesticus* alleles at assayed loci. Sage et al. (1986a) and Moulia et al. (1991) assayed respectively four and 10 enzyme loci for this purpose. However, the interval "hybrids" occupy on this scale differs from study to study. Expressing the *HI* as percentage *domesticus*, "hybrids" have $12.5\% < HI < 87.5\%$ in Sage et al. (1986a), $20\% < HI < 60\%$ in Moulia et al. (1991), and $2\% < HI < 97\%$ in Moulia et al. (1993).

Regarding the original wormy mouse study, Klein (1988) pointed out that carefully designed sampling was necessary before it could be concluded that parasites play a role in the outcome of house mouse hybridization. We would add to this a second note of caution. From the first, these studies implicitly assume parasite load as a proxy for fitness. This appears a dangerous assumption when considering hybrids because (1) the fitness of a sterile hybrid is zero no matter what its parasite load; (2) fit hosts can be either resistant or tolerant (Raberg et al. 2007)—a tolerant individual maintains high fitness regardless of its parasite load. Taking parasite load as a proxy for fitness assumes a strong correlation between the two. The two points above illustrate why this is questionable in general and particularly in a hybrid zone.

These weaknesses and inconsistencies in data and interpretation motivated us to examine parasite load and individual parasite diversity at 107 localities across the Czech–Bavarian portion of the HMHZ in a 145 × 50 km belt, using a well-designed sampling effort. We focus on helminth parasites for comparison with the previous studies, and because evidence continues to accumulate that variation for helminth resistance/susceptibility has a strong genetic component in mice (Wakelin 1988; Behnke et al. 2003). Rather than dividing individuals into arbitrarily constructed pure/hybrid categories, we consider each individual in relation to its hybrid index based on 1401 diagnostic SNPs, and develop a model-based analysis of mice characterized by these hybrid indices. Two contrasting genotype-based predictions regarding parasite load and diversity in hybrids are (1) low load/diversity due to hybrid resistance resulting from the new combinations

Figure 1. The European house mouse hybrid zone. The black line depicts the course of the zone. The white arrows indicate previously studied transects and the rectangle indicates the position of the studied area.

of genes brought together in the hybrid genotype, including increased heterozygosity; and (2) high load /diversity due to hybrid susceptibility, resulting from the breakup of coadapted gene complexes involved in parasite interactions in the new combinations of genes brought together in the hybrid genotype (Sage et al. 1986a; Moulia et al. 1991). Both resistance and susceptibility are expected to increase with the degree to which new combinations of genes have been brought together in the hybrid genotype of an individual. A model explicitly allowing for either possibility allows us to address the question of whether parasite load and diversity is more consistent with hybrid resistance or susceptibility in a clear hypothesis-driven framework. Separate load analyses over helminth species infesting different parts of the host allow us to gauge the generality of our conclusions. Randomization of loads over localities allows us to measure false positive rates due to load heterogeneity.

Material and Methods

SAMPLING

From 2006 to 2009, a total of 689 mice (2006: $N = 124$; 2007: $N = 243$; 2008: $N = 130$; 2009: $N = 192$) were trapped with metal and wooden live traps at 107 localities scattered across a belt 145-km long and 50-km wide stretching from northeastern Bavaria (Germany) to western Bohemia (Czech Republic) (Fig. 1). Parasite community and load can fluctuate seasonally (Haukisalmi et al. 1988; Abu-Madi et al. 2000), and so to minimize seasonal effects we sampled in the same period each year (10 days centered on the end of September). All trapped mice were euthanized with halothane and dissected the day after capture (with the exception of 26 mice in 2006, 10 days after capture). Mouse tissue samples were collected in ethanol and liquid nitrogen for molecular genotyping.

PARASITES

The mouse abdominal cavity was inspected for parasites, and the liver and digestive tract were directly dissected under a binocular microscope. Helminths were isolated and stored in 70% ethanol for later microscopic identification by comparison with the original species descriptions. All helminth species were counted during dissection and stored in separate tubes except the visually very similar pinworm species, *A. tetraptera* and *Syphacia obvelata*. For mouse ceca highly infected by pinworms (> 100 worms), only 100 pinworms were collected and the total number was estimated using a graduated petri dish. The identification of helminth species was performed by A.R.

HOST GENOTYPING

The mice in this study were genotyped at 1401 diagnostic X-linked and autosomal SNP markers spaced approximately 1.86 Mb apart across the genome as part of a parallel study (for more details on the molecular protocols and choice of SNPs, see Wang et al. 2011). The *HI*, georeference and parasite load for each individual is made available in Supporting Information Table S1.

DATA FROM THE LITERATURE

Because one goal was to compare our results to the two previous studies on helminth parasites in the HMHZ, we wished to apply our model-based analysis to the original datasets of Sage et al. (1986a) and Moulia et al. (1991). We collected data from Figure 3 of Sage et al. (1986a), which represents nematode load in relation to individual *HIs* based on four enzymatic loci (hereafter the Sage dataset). Ninety percent of these nematodes were pinworms. Unfortunately, Moulia et al. (1991) provide no data for individual *HIs*.

ANALYSES

Total parasite prevalence and abundance

We used Quantitative Parasitology version 3.0 (QP3.0) to estimate parasite prevalence (proportion of infected hosts among all the hosts examined) and mean load (the mean number of parasites in all hosts, including the zero values of uninfected hosts) with 95% confidence limits over the complete dataset (Rózsa et al. 2000). Confidence intervals for prevalence and load were estimated using Sterne's Exact method (Reiczigel 2003) and bootstrap (1000 replicates), respectively. Using QP3.0, we modeled the aggregation of parasites within hosts using the negative binomial distribution, calculating maximum likelihood estimates (MLEs) of parameter k (Bliss and Fisher 1953). QP3.0 also calculates a χ^2 goodness of fit between expected and observed frequencies under the negative binomial model. The pinworm species, *A. tetraptera*

and *S. obvelata*, are considered together throughout all the analyses.

A model of hybridization effects on parasite load in a hybrid zone

Fritz and colleagues lay out a framework considering genetic effects on parasite load (see Figs. 1 in Fritz et al. 1994 and Fritz 1999), and this has formed a basis for further discussion (Moulia 1999; Wolinska et al. 2008). This framework is, however, limited: first, only F1s and first-generation backcrosses are considered; second, although deviations from additivity are considered, these deviations are limited to dominance effects, rather than epistatic effects, and so the scenarios are implicitly limited to a single locus of large effect (or a highly unlikely scenario where multiple loci have the same polarity of dominance). Here, we wish to consider both individual load for specific parasites, and individual diversity—the number of parasite species co-occurring within one host. We require an individual-based model allowing for hybridization effects on parasite load and diversity (hybrid resistance vs. susceptibility) that increase with the degree to which new combinations of genes have been brought together in the hybrid genotype of an individual. Barton's concordance (Szymura and Barton 1986; Macholán et al. 2011) provides the basis for our model. Briefly, Barton's approach characterizes individuals by a hybrid index HI, and models locus specific effects as a function of the individual's expected heterozygosity $H_e = 2\,HI\,(1 - HI)$, which is zero for the purest individuals and maximal (1/2) when half the genome is estimated to come from each source. We use H_e as our expectation for the degree to which new combinations of genes have been brought together in the hybrid genotype of an individual. Within loci, H_e informs us about how often alleles of different source are brought together. The same is true across loci, as Fisher's junctions between DNA strands of different source accumulate in proportion to H_e (Baird 2006). It should be noted that H_e, being a summary statistic, does not allow some sets of genotypes to be distinguished. For example, an F1 will have the same H_e as an individual with half their loci homozygous for alleles from one species, and half for the other. However, no F1s or early-generation backcrosses have been detected in this zone despite extensive sampling (Macholán et al. 2007), and the correlation between H_e and observed heterozygosity across hybrid individuals is strong ($r^2 = 0.88$, $P < 0.0001$), making it likely that H_e captures the information we desire: summarizing how hybrid an individual is in the HMHZ. In the absence of hybridization effects, we model a parasite load or diversity trait L as changing linearly across the hybrid index from its expectation L_1 in one taxon to L_2, its expectation in the other taxon. The expected load or diversity for an individual with hybrid index HI is then

$$L(HI) = L_1\,(1 - HI) + L_2 HI. \qquad (1)$$

Here, the implicit assumption is the genetic component influencing the trait is well described by the infinite sites model of quantitative genetics, with additive genetic variance. Hybridization effects are introduced as deviations from this additive model, in proportion to H_e. The trait expectation for an individual, allowing for a hybridization effect of magnitude V, is then

$$L(HI, V) = Max\,[L(HI) - V H_e, \partial]. \qquad (2)$$

Here, a positive hybridization effect will reduce parasite load or diversity, such that $+V$ equates with our notion of hybrid resistance. A negative effect will increase load or diversity and hence $-V$ equates with our notion of hybrid susceptibility. The trait expectation cannot be reduced below load/diversity level ∂ no matter how high the resistance. Trial results were checked for invariance to choice of $0.001 < \partial < 0.01$ and $\partial = 0.01$ was chosen for further analyses. For zero hybrid effect, the model reduces to the additive expectation (1). Our modeling approach is analogous to a General Linear Model (GLM) with second-order polynomial effect in HI, coefficients $\{L_1, L_2 - L_1 + 2V, -2V\}$. When V is constrained to be zero, the model becomes first order in HI. Equation (2) provides an individual-based expectation for parasite load/diversity, but does not tell us how individual's loads/diversities are distributed around this expectation. The situation is simplest for individual parasite diversity, because observed distributions usually match expectations for a random assemblage of the species present (Poulin 1997), often modeled using the Poisson distribution (Goater et al. 1987), and therefore requiring no further parameter than the expectation. Regarding load, however, macroparasites generally exhibit aggregated distributions, that is they cluster within individual hosts (Grenfell et al. 1995; Shaw and Dobson 1995; Shaw et al. 1998), this aggregation being well described by the negative binomial distribution (Crofton 1971; Shaw and Dobson 1995). As the exponential parameter of the negative binomial distribution (denoted k) tends to infinity, the distribution tends to the Poisson distribution, that is the distribution for zero aggregation. We therefore parameterize aggregation as $A = 1/k$, so as A tends to zero, aggregation tends to zero. This means the Poisson distribution model for parasite diversity can be thought of as a special case ($A = 0$) of the more general negative binomial model. For parasite load, the aggregation parameter A is expected to be study-specific, depending on the density of both hosts and parasites, and the nature of their interaction. As with the previously considered load/diversity traits (eq. 1), we could assume additive change in A across the hybrid index from its expectation A_1 in one taxon to A_2 in the other taxon. However, the mouse tension zone may be located at a trough in host density or a barrier to host dispersal, both of which might affect the aggregation of the parasites they carry. We therefore allow for deviation Z from this additive model,

in proportion to H_e, which is maximal at the zone center

$$A(HI) = A_1(1 - HI) + A_2 HI$$
$$A(HI, Z) = Max[A(HI) + ZH_e, 0). \quad (3)$$

Here, a positive zone center deviation $+Z$ will increase the expected aggregation, and the aggregation parameter has a natural lower bound at $A = 0$, where the negative binomial distribution simplifies to the Poisson. Given expectations for a load/diversity trait and its aggregation, the likelihood of an individual with hybrid index HI and observed load/diversity L_{obs} is then proportional to

$$PDF(NegativeBinomial[L(HI, V), A(HI, Z)], L_{obs}). \quad (4)$$

Here, *PDF* stands for probability density function, and the *NegativeBinomial* distribution is parameterized by two arguments: its expectation, and the inverse of its exponential parameter, respectively. The likelihood for a set of independent observations from a hybrid zone is proportional to the product of these individual based likelihoods, and this allows us to estimate the parameter vector $\phi\{V, L_1, L_2, A_1, A_2, Z\}$ in the likelihood framework. The hybrid resistance parameter V is the focus of inference, the remaining parameters being of only taxon- or zone-specific interest, although all are necessary to make sound inference. Likelihood maximization and support exploration used the FindMaximum function of Mathematica (Wolfram Research, Inc. 2010). Convergence was checked from multiple points in the parameter space.

The likelihood framework allows straightforward comparison of nested hypotheses over the same total dataset using *G*-tests. For example, to detect if there are significant sex differences in the dataset, the maximum log likelihood of ϕ over all observations, $ML(\phi)$, can be compared to the total of $ML(\phi_F)$ over female observations and $ML(\phi_M)$ over male observations. Allowing each sex to have its own set of parameters will tend to increase the total likelihood, and the *G*-test allows us to decide whether any increase is significant. Our inference approach is to compare a series of nested hypotheses, working from simple toward more complex, and accepting more complex hypotheses only when justified by their improvement in the likelihood of the data. The hypotheses place different constraints on the underlying variation in parasite load. The choice of hypotheses reflects the major sources of variation in mouse parasite load: taxon and sex specific (Wilson et al. 2002) that may potentially obscure hybridization-specific variation. H_0: the expected load for the subspecies and between sexes is the same. H_1: the mean load across sexes is the same, but can differ across subspecies. H_2: the mean load across subspecies is the same, but can differ between the sexes. H_3: the mean load can differ both across subspecies and between sexes.

Estimating false positive rates due to load heterogeneity across sampling localities

Although many localities were sampled, it is possible that parasite heterogeneity by chance falls across localities in a pattern matching the places most hybrids are found. This could result in false positives for hybrid-specific effects in the individual-based analyses described above. We estimated these false positive rates by randomizing locality estimates of load and diversity and asking how often we observed similar or greater hybrid-specific effects. To capture local parasite heterogeneity, load and diversity were estimated at the locality level for all localities with greater than 10 individuals (> 20 localities in all cases, spread throughout the field area: Fig. 2). Every locality was then assigned a new load or diversity expectation drawn at random with replacement from this captured heterogeneity list. Parasite loads and diversity were then simulated for the individuals at each locality, according to the expectations randomly assigned to that locality. The GLM equivalent of the individual-based likelihood analyses (above) was then run on the simulated dataset. False positive rates in 10,000 such simulations for each trait were run in 64-bit R 1.4 for MacOS using glm.nb from the MASS package (version 7.3–12) for GLM with negative binomial error distribution (R Development Core Team 2011).

Results

PARASITE DIVERSITY, LOAD, AND AGGREGATION IN THE HMHZ

In total, 10 helminth species were found across the transect region: four cestodes and six nematodes, infesting various parts of the mouse (see Table 1). The most abundant parasites were the caecal nematode pinworms (*A. tetraptera* and *S. obvelata*): 70.9% of mice were infected, with an average load of 39.2 pinworms per mouse (Table 1, Fig. 2C). The second most widespread infection was due to the caecal nematode *Trichuris muris* (whipworm) found in 21.1% of mice with an average load of 3.9 individuals per mouse (Fig. 2D). The larval stage of *Taenia taeniaeformis* (tapeworm) was the most prevalent cestode, found in the liver of 10.7% of individuals (Fig. 2E). The fourth most prevalent parasite was *Mastophorus muris*, found in the stomach of 9.4% of individuals (Fig. 2F). Four helminth species were rare (prevalence < 5%): *Calodium hepaticum* was only found in one mouse and *Heterakis spumosa*, *Mesocestoides* sp., and *Hymenolepis diminuta* infections were restricted to a few localities. The negative binomial distribution seems to describe parasite load well for all parasites (Table 1, Fig. S1). In two cases, tapeworms and *Rodentolepis microstoma*, observations differ significantly from negative binomial expectations, but removal of a single high-load outlier (in each case an almost pure *domesticus* individual: $HI = 0.98$ and

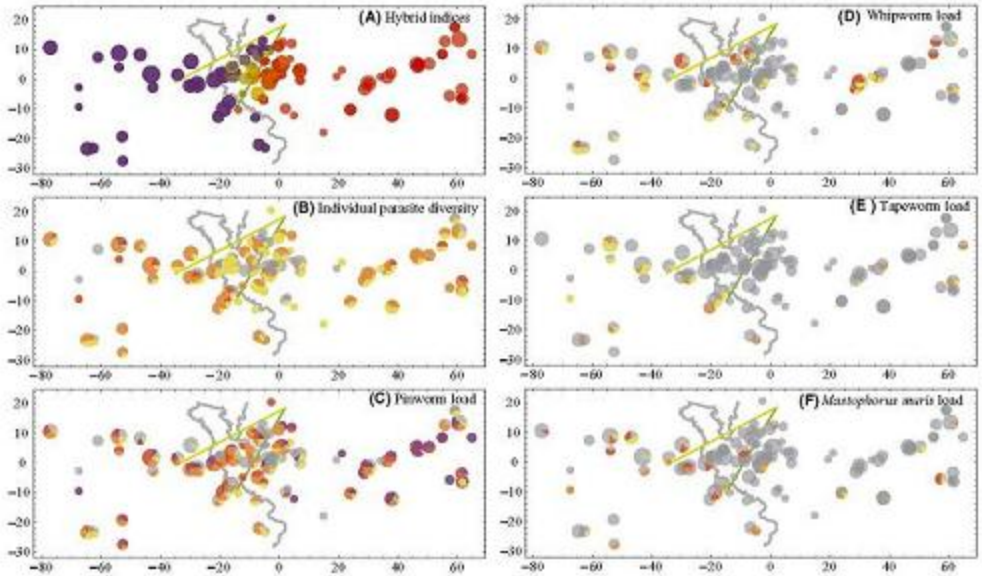

Figure 2. Geography of hybrids and parasites across the Czech–Bavarian region of the European house mouse hybrid zone. Thick gray line = Czech–German border (northeastern Bavaria to the west, western Bohemia to the east). Data for 107 localities are shown (axes in km, after gnomonic projection onto the plane around their centroid). The consensus center of the hybrid zone is shown in green, and the extent of the Y introgression is indicated by the dashed yellow line, following Macholán et al. (2008). Pie areas are proportional to the $\log_{10}$ of (locality mouse sample sizes + 1).

(A) Distribution of individual's hybrid indices (*HI*s) within localities. Colors are taken from a continuous blend of blue (*domesticus*) through yellow (*HI* = 0.5) to red (*musculus*). (B) Distribution of parasite diversity over individuals within localities. Gray: individuals with no parasites, yellow: 1, orange: 2–3, red: 4–5, purple: 6–8 parasite species. (C–F) Distribution of individual's parasite load for localities. Load bins (A–B) are designed to divide nonzero expectations equiprobably, relative to the (H_0) maximum likelihood estimate parameterization for the negative binomial distribution (see Table 2). Gray: individuals with none of the focal parasite. Yellow, orange, red, purple: Load bins [1–4], respectively. (C) Distribution of pinworm load over individuals within localities. Load bins: [1–4], [5–23], [24–83], [> 83]. (D) Distribution of whipworm load over individuals within localities. Load bins: [1–5], [6–20], [> 20]. (E) Distribution of tapeworm load over individuals within localities. Load bins: [1], [> 1]. (F) Distribution of *Mastophorus muris* load over individuals within localities. Load bins: [1], [2–6], [> 6].

HI = 0.93, respectively) makes the difference from expectations nonsignificant (Table 1).

HYBRIDS AND PARASITISM IN THE MOUSE HYBRID ZONE

Figure 2A shows the distribution of individual's hybrid indices (*HI*)s within localities. The number of free parameters and the outcomes for likelihood analyses of the load/diversity of each and all individuals with respect to the four nested hypotheses are shown in Table 2. Allowing for potential differences in load/diversity between male and female mice (H_2, H_3) and pure mice of the two taxa (H_1, H_3), ensures any perceived pattern of hybridization effects is not due to these potentially confounding factors. The final columns of Table 2 present the drop in likelihood for each hypothesis if the hybrid resistance parameter V (deviation from additivity) is fixed at zero. Table 3 shows the significance of any gains in likelihood as hypotheses become more complex.

Individual parasite diversity

Although H_1 is significantly favored over H_0, no further increase in hypothesis complexity is justified (ΔLLH_1–H_0 = 7.45, $P < 0.0001$; Table 3). That is, the significantly favored model allows for greater parasite diversity in *domesticus* than *musculus*, sex effects being nonsignificant. The best estimate of the resistance parameter for H_1 is positive ($V = +1.15$; Table 2) and significantly greater than zero (if V is fixed at 0, the log likelihood of the data

Table 1. Prevalence, mean load, parameter *k* of the negative binomial distribution, and characteristics of the helminth parasites in the house mouse hybrid zone.

	Species	*N* mice	Site of infection	Specificity	Prevalence Ni (%) [CI 95%]	Mean load (Max) [CI 95%]	*k*
Cestodes	*Taenia taeniaeformis*	689	Liver	Generalist	74 (10.7%) [8.62–13.26]	0.23 (42) [0.15–0.45]	0.147*[1]
	Mesocestoides sp.	689	Peritoneal cavity	Generalist	12 (1.7%) [9.8–30.2]	0.09 (36) [0.02–0.30]	0.007*
	Hymenolepis diminuta	689	Intestine	Specific to Murinae	4 (0.6%) [0.20–1.49]	0.02 (6) [0.00–0.05]	-
	Rodentolepis microstoma	689	Intestine	Specific to Murinae	55 (8.0%) [6.15–10.28]	0.48 (31) [0.34–0.71]	0.030*[2]
Nematodes	*Calodium hepaticum*	689	Liver	Generalist	1 (0.1%) [0.02–0.83]	-	-
	Mastophorus muris	689	Stomach	Generalist	65 (9.4%) [7.45–11.88]	0.68 (75) [0.47–1.08]	0.032*
	Trichuris muris	682	Caecum	Specific to Murinae	144 (21.1%) [18.17–24.32]	3.9 (200) [2.93–5.44]	0.056*
	Heterakis spumosa	680	Caecum	Specific to Murinae	28 (4.1%) [2.85–5.93]	0.46 (84) [0.26–0.89]	0.011*
	Pinworms (*Aspiculuris* + *Syphacia*)	680	Caecum + colon	Specific to mouse	482 (70.9%) [67.37–74.20]	39.19 (693) [33.68–45.97]	0.250*

[1]The individual (*HI* = 0.98) with 42 *T. taeniaeformis* cysts was excluded for the estimation of *k*. [2]The individual (*HI* = 0.93) with 31 *R. microstoma* was excluded for the estimation of *k*. We tested how the negative binomial distribution as a theoretical model fit the observed data with the χ^2 test. H_0: the negative binomial distribution represents the observed data as a theoretical model. *Observed and expected frequencies do not differ significantly ($P = 0.05$) so there is no statistical evidence to reject H_0.

drops 21.81 units, $P = 3.97 \times 10^{-11}$). Thus, the best model of parasite diversity across the hybrid index has a significant deviation from additivity: *domesticus* mice have higher parasite diversity compared to *musculus*, and hybrids have significantly reduced parasite diversity compared to additive expectations (cf. Fig. 2B). Figure 3A shows the MLE of parasite diversity in hybrids in fact drops lower than that in either mouse taxon. Of the 10,000 simulations randomizing heterogeneity in parasite diversity over localities, none showed a hybrid effect as or more significant than the individual-based estimate.

Parasite load

The four most common parasite species were selected for load analysis: in order of prevalence, the pinworms, the whipworms, the tapeworms (without the *domesticus* outlier individual), and *M. muris* (Fig. 2 C–F). For the pinworms, H_1 is significantly favored over H_0 with no further increase in hypothesis complexity justified (ΔLLH_1–$H_0 = 8.31$, $P = 0.0002$). The best estimate of the resistance parameter is positive ($V = +1.39$) and significantly greater than 0 (LL drop if V fixed to 0 equals 6.67, $P = 0.0003$). The best model of pinworm load across the hybrid index has a significant deviation from additivity: pinworm load is higher in *musculus* compared to *domesticus*, and hybrids have significantly lower load compared to additive expectations. Figure 3B shows the MLE of pinworm load in hybrids, as with diversity, drops lower than that in either mouse taxon. Of the 10,000 simulations randomizing heterogeneity in pinworm load over localities, 71 showed a hybrid effect as or more significant than the individual-based estimate (false positive rate 0.0071). For the whipworms, H_1 is significantly favored over H_0 (ΔLLH_1–$H_0 = 6.3$, $P = 0.002$) and a further increase in hypothesis complexity is justified with H_3 significantly better than H_1 (ΔLLH_3–$H_1 = 6.98$, $P = 0.03$) reflecting a taxon × sex interaction. The estimate of the resistance parameter is significantly positive for H_1 ($V = +2.26$, $P = 0.009$) and H_3 shows this is driven by significant male effects ($V = +2.38$, $P = 0.002$ for males and $V = +0.54$, $P = 0.86$ for females). Thus, the best model of whipworm load across the hybrid index has a significant deviation from additivity with a taxon × sex interaction: male whipworm load is lower in *domesticus* than *musculus*. Female whipworm load is lower in *musculus* than *domesticus*. Hybrid males have significantly lower load compared to additive male expectations (Fig. 3C). Of the 10,000 simulations randomizing heterogeneity in whipworm load

Table 2. Parameter estimates of the explicit models for individual parasite diversity, load for the four most prevalent parasites of the mouse hybrid zone, and Sage dataset.

	N	LL	$L_1(\sigma)$	$L_1♀$	$L_2(\sigma)$	$L_2♀$	$A_1(\sigma)$	$A_1♀$	$A_2(\sigma)$	$A_2♀$	$V(\sigma)$	$V♀$	$Z(\sigma)$	$Z♀$	LLdropV(σ)–>0	LLdropV♀–>0
Individual parasite diversity																
H_0	2	−922.28	1.92								1.19				−24.37***	
H_1	**3**	**−914.83**	**1.61**		**2.17**						**1.15**				**−21.81*****	
H_2	4	−921.54	1.82	2.01							1.05	1.30			−8.32***	−16.14***
H_3	6	−913.45	1.67	1.53	1.98	2.30					1.05	1.21			−8.33***	−12.48***
Pinworm load																
H_0	4	−2670.00	53.21				3.72				1.43		0.18		−8.46***	
H_1	**6**	**−2661.69**	**73.99**		**33.49**		**3.60**		**3.62**		**1.39**		**0.43**		**−6.67*****	
H_2	8	−2669.12	56.20	50.27			4.07	3.47			1.45	1.40	−0.32	0.54	−4.80**	−3.77**
H_3	12	−2659.00	68.83	79.79	42.24	27.07	3.86	3.41	3.85	3.47	1.44	1.34	−0.17	0.84	−4.22**	−2.84*
Whipworm load																
H_0	4	−834.22	6.61				10.69				2.26		2.06		−3.63**	
H_1	**6**	**−827.92**	**4.22**		**8.64**		**16.35**		**16.41**		**2.26**		**2.03**		**−3.44****	
H_2	8	−832.84	5.64	6.03			10.52	10.66			2.38	1.30	1.90	2.14	−4.85**	-0.11^{NS}
H_3	**12**	**−820.94**	**6.09**	**1.19**	**5.15**	**8.45**	**11.58**	**25.59**	**11.60**	**25.69**	**2.38**	**0.54**	**1.90**	**2.10**	**−4.79****	**-0.02^{NS}**
Tapeworm load																
H_0	4	−270.20	0.32				2.19				2.36		3.97		-0.39^{NS}	
H_1	**6**	**−244.95**	**0.07**		**0.54**		**21.22**		**22.11**		**2.60**		**4.00**		**-0.36^{NS}**	
H_2	8	−267.52	0.16	0.39			1.70	2.03			−3.79	3.03	3.96	4.43	-0.19^{NS}	-0.73^{NS}
H_3	12	−242.24	0.05	0.09	0.41	0.58	942.21	21.10	943.65	21.91	0.55	3.04	3.99	4.44	0.01^{NS}	-0.79^{NS}
Mastophorus muris																
H_0	4	−390.44	0.89				23.30				1.23		1.31		-0.77^{NS}	
H_1	**6**	**−383.36**	**0.21**		**1.45**		**33.35**		**33.38**		**1.12**		**1.29**		**-0.59^{NS}**	
H_2	8	−389.48	0.91	0.77			22.96	23.96			1.46	0.46	0.78	1.61	-1.13^{NS}	-0.01^{NS}
H_3	12	−382.05	0.19	0.20	1.66	1.07	27.1	43.46	27.12	43.49	1.46	−0.21	1.01	1.51	-1.1^{NS}	0^{NS}
Nematode load (Sage et al. 1986a)																
H_0	4	−465.91	44.21				2.97				−8.03		−0.42		−6.79***	
H_1	6	−463.78	68.97		26.53		3.12		3.21		−7.41		−0.19		−4.64**	

N, number of parameters; LL, log likelihood; L_1, parasite load *musculus*; L_2, parasite load *domesticus*; A_1, aggregation *musculus*; A_2, aggregation *domesticus*; V, hybrid resistance parameter; Z, parameter allowing aggregation to change in the zone center; LLdrop V–>0, drop in likelihood if V is fixed to zero. If sexes are separated (H_2 and H_3), $L_1(\sigma)$, parasite load of *musculus* males, $L_1♀$, parasite load of *musculus* females and so on. ^{NS}Nonsignificant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. H_0: the mean load across subspecies and between sexes is the same. H_1: the mean load across sexes is the same, but can differ across subspecies. H_2: the mean load across subspecies is the same, but can differ between the sexes. H_3: the mean load can differ both across subspecies and between sexes. In bold, models significantly favored.

Table 3. Significance of the *G*-tests comparing the log likelihood of the nested hypotheses.

	H_1 versus H_0	H_2 versus H_0	H_3 versus H_1	H_3 versus H_2
Individual parasite diversity	**0.0001**	0.4772	0.4301	**0.0003**
Pinworms	**0.0002**	0.7801	0.4967	**0.0004**
Whipworms	**0.0018**	0.6000	**0.03**	**8.75×10^{-05}**
Tapeworms	**1.09×10^{-11}**	0.2537	0.4896	**2.74×10^{-10}**
Mastophorus muris	**0.0008**	0.7515	0.8529	**0.005**
Sage dataset	0.1181			

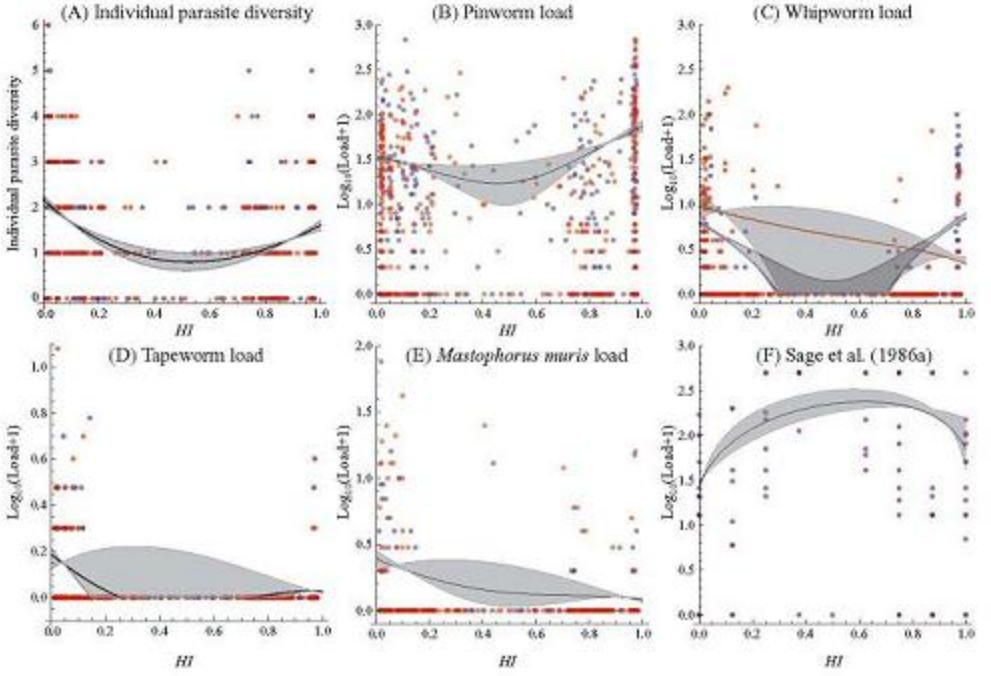

Figure 3. Distributions of individual parasite diversity and mean load with two-unit support envelopes across individual *HI*s compared to the ML parameterization of the best model: (A) Individual parasite diversity (model H_1); (B) Pinworms (model H_1); (C) Whipworms (model H_3); (D) Tapeworms (model H_1); (E) *Mastophorus muris* (model H_1); (F) Sage dataset (model H_1). *HI* ranges from *HI* = 0 (*domesticus*) to *HI* = 1 (*musculus*). Blue: males, red: females, purple: no sex distinction. The scale of the y-axis is individual parasite diversity for (A) and $\log_{10}$ (load + 1) for the remaining figures. The two-unit support envelopes show that there is increased uncertainty of mean load for the most hybrid individuals, corresponding to the simple fact that F1s and equal-source-contribution hybrids are very rarely observed in the European mouse hybrid zone. For whipworm load (C), mean loads for males and females are shown separately because of the significant taxon × sex interaction (see text).

over localities, 96 showed a hybrid effect as or more significant than the individual-based estimate (false positive rate 0.0096). Finally, for the tapeworms and *M. muris*, H_1 is highly significantly favored over H_0 with no further increase in hypothesis complexity justified (ΔLLH_1–$H_0 = 25.24$, $P = 1.09 \times 10^{-11}$ and ΔLLH_1–$H_0 = 7.07$, $P = 0.0008$, respectively). Although the estimates of the resistance parameters are positive ($V = +2.6$ and $V = +1.12$, respectively), there is insufficient power to exclude the zero V possibility (LL drop if V fixed to 0 equals –0.36, $P = 0.39$ and –0.59, $P = 0.28$). Thus, the best models of tapeworm and *M.*

muris loads across the hybrid index do not significantly deviate from additivity with tapeworm and *M. muris* load estimates eight- and sevenfold higher in *domesticus* than in *musculus*, respectively (Table 2 and Fig. 3D, E).

In summary, the estimate of the resistance parameter V is positive for all cases and significantly greater than zero for individual parasite diversity, pinworm load, and whipworm load (Table 2). That is we find "hybridization effects consistent with hybrid resistance and in contradiction to hybrid susceptibility." Although the lower prevalence parasites (the tapeworms and *M. muris*) further support this pattern, each individually holds insufficient information to distinguish the positive V MLE from the $V = 0$ case. It should be noted that, if in reality hybrids were susceptible to hyperparasitism morbidity, this would remove all but the least parasitized hybrid mice from our trapping observations. This would give us a signal of reduced parasite load in trapped hybrids. We can discount the possibility of such strong viability selection due to a parallel study of the age structure of mice (J. Goüy de Bellocq and S. J. E. Baird, unpubl. data): there is no significant difference in the distribution of old versus young mice across the hybrid index.

Although our results are consistent with hybrid resistance, an alternative explanation is that a patch of low parasitism (across multiple parasites) has coincidentally fallen at the center of the hybrid zone. Hybrid resistance would imply a within-locality correlation between hybridness and parasitism, and tests for such a correlation within localities would avoid potentially confounding environmental variation. However, mouse *HI* varies little within localities in our dataset, giving within-locality tests so little power there is no chance of detecting any effects. The probability of the coincidental patch explanation, however, can be quantified to some extent: If patches of low parasite diversity such as the central gray area in Fig. 2B occur throughout the potential field area, what is the probability of one falling "just so," such that it would give reduction in hybrid parasitism of the same magnitude or greater than we have observed? This concern is addressed through simulation in the Appendix S1: When such patches fall at random, the mean and mode for hybrid effect V is zero, and the frequency with which V matches or exceeds our observations is 0.044. This test is conservative, as the probability falls further when smaller or larger patch size is simulated (results not shown): It seems unlikely that random patches would not only fall "just so" in the right place, but also "just so" at the right size, to mimic our results.

COMPARISON WITH A PREVIOUS STUDY

The distribution of the Sage dataset nematode load across all individuals differs significantly from ML negative binomial expectations (Fig. S1F). Because host gender was not available for this dataset, we could only evaluate hypotheses H_0 and H_1. H_0 shows V significantly different from zero (H_0: $V = -8.03$; if no hybrid effect, $\Delta LL = -6.79$, $P = 0.0002$), that is allowing a hybrid effect significantly increases the likelihood of the observations. Although we know these 93 individuals come from 22 localities, we cannot reconstruct which individuals come from which locality, and so we cannot test for the false positive rate due to locality heterogeneity. In contrast to our dataset, the estimate of V is negative, consistent with hybrid susceptibility (Fig. 3F). Again contrary to our dataset, where a significant taxon effect was found for each parasite type, adding parameters for a taxon effect was not justified for the Sage dataset (ΔLLH_1–$H_0 = 2.14$, $P < 0.12$).

Discussion

We ask whether hybrid resistance or susceptibility best explains the parasite load of hybrids between two subspecies of mice. We find that allowing nonadditive effects significantly increases the likelihood of the observations for the two most prevalent types of helminths, the pinworms and the whipworms, and also for the overall helminth diversity in individuals. In each case, these effects were consistent with hybrid resistance, and contradictory to hybrid susceptibility. Our results are robust to potential confounding factors (sex- and taxon-specific loads, load heterogeneity over localities). Loads for the two less-prevalent parasites (tapeworms and *M. muris*) further support hybrid resistance, but individually cannot be shown significantly different from zero, as expected given less power due to less parasites. In all cases, we find significant taxon differences. Our results are consistent with experimental infection of *domesticus* and *musculus* mouse strains and their hybrids (from F1 to F4) in controlled conditions with the pinworm *A. tetraptera* (Derothe et al. 2004), which showed either significantly reduced load (F1, F3, F4) or no significant effect (F2).

Our results are in contradiction with two previous field studies of the same biological model, which found increased load in hybrids relative to parentals, and concluded this was due to the breakup of coadapted gene complexes (Sage et al. 1986a; Moulia et al. 1991). Reanalyzing the Sage dataset, we find that the deviation from additivity for hybrids is very significantly negative, consistent with hybrid susceptibility. We find no evidence for a taxon difference in Sage's data, and the distribution of parasite load across individuals is very different from the negative binomial distribution commonly reported in the literature and consistent with all parasites we have analyzed (Figs. 3F, S1F).

WHY DOES OUR PATTERN OF HELMINTH INFECTION DIFFER FROM THE TWO PREVIOUS FIELD STUDIES?

The contrast between hybrids and parentals with respect to parasites is the key difference between studies. Moving across the mouse hybrid index from one subspecies through hybrids to the

other subspecies, the two previous studies (Sage et al. 1986a, Moulia et al. 1991) found a pattern of low-high-low parasite load. Our study finds a high-low-high pattern. Assuming both patterns are well supported, explanations for this "heterogeneity over studies" will revolve around parasite heterogeneity, host heterogeneity, or some combination of the two. The two previous studies conclude that the low-high-low pattern is due to the genetics of the host: specifically that hybrids are hyperinfected because they are hybrids. The heterogeneity over studies might then be explained by proposal A: "hybridization gives rise to susceptible individuals in one part of the hybrid zone, but resistant individuals in another part (or at another time)." Proposal A cannot be refuted with the current data and, if accepted, at least partially rescues the conclusions of the previous studies. However, it severely undermines the suggestion that parasites play an important role in the maintenance of the mouse species barrier (Sage et al. 1986a; Moulia et al. 1991) because for the helminth role to be globally important, their effects should be geographically consistent.

We find proposal A unsatisfactory because it requires that the contrast between hybrids and parentals with respect to their helminth parasites is radically altered in different parts of the zone (or at different times). The HMHZ is broadly similar over a very long front (> 2500 km) despite the extremes having quite different times of initial contact (Macholán et al. 2007). While Teeter et al. (2010) speculate the zone might have different mechanisms maintaining it in different places, Macholán et al. (2011) point out that the differences in observations motivating this speculation are very likely due to different sampling strategies, concluding "there is some evidence of common architecture of reproductive isolation, and no reliable evidence to the contrary." Occam's razor would suggest the contrast between hybrids and parentals with respect to their parasites is similar along this front. Further, host-specific direct life cycle parasites such as the pinworms are likely to have a history of close coevolution with their hosts (Hugot 1988), and thus seem unlikely to preferentially infect hybrids in one part of the zone, while preferentially avoiding hybrids elsewhere.

It has been pointed out that hybrid study systems repeatedly screened for infection often show variable infection scenarios (Wolinska et al. 2008). Wolinska and colleagues proposed that (hereafter proposal B) inconsistent patterns of infection observed over space and time in hybridizing communities may be due to frequency-dependent selection, that is Red Queen dynamics, but extrapolated from the level of alternative genotypes to the level of alternative taxa (Wolinska et al. 2006, 2008). The core of proposal B is "the dynamic scenario predicts continuous, bidirectional changes in the relative infection of hybrid and parental taxa. The change is expected to result from frequency-dependent selection against common genotypes contained within each of the taxa" (Wolinska et al. 2008, p. 124). The relevance of this model to systems other than *Daphnia* (Wolinska et al. 2006), its inspiration, is however unclear. Although common host genotypes might be expected to occur in parental species, the notion of hybrids as a taxon with common genotypes is restricted to systems where only F1s (and perhaps early backcrosses) are produced, and where, for example, clonal reproduction can maintain such hybrid "taxa." In contrast, the defining feature of post-F1 hybridization (the HMHZ case) is an explosion of new possible genotypes (2 raised to the power of L, the number of loci considered). As L increases, every hybrid genotype quickly becomes rare, making the taxon "hybrids" an extremely unlikely target for parasites in search of common host genotypes. We do not, therefore, think this kind of Red Queen model could drive heterogeneity of the hybrid–parasite interaction along the HMHZ.

Having assumed first, that both reported hybrid parasitism patterns are well supported and, second, that hybrids were hyperinfected in previous studies because they were hybrids, the heterogeneity over studies leads us to unsatisfactory and seemingly overcomplicated proposals A and B regarding the hybrid–parasite interaction. This leads us to question our initial assumptions: are both hybrid parasitism patterns equally supported?

Quite apart from the order-of-magnitude increase in mice, parasites, localities, and loci sampled in our study compared to the previous studies, and the laboratory results demonstrating hybrid resistance rather than susceptibility, there are further reasons to suggest the contrasting patterns of hybrid parasitism between studies are not equally supported. If the spatiotemporal heterogeneity of parasite load is expected to be highly stochastic (Haukisalmi et al. 1988; Montgomery and Montgomery 1989; Behnke et al. 1999), careful sampling design becomes crucial if we wish to distinguish local heterogeneity effects from hybridization effects. This sampling issue regarding the original wormy mouse study was first pointed out by Klein (1988). Differences between the sampling design of the previous and current studies should therefore be considered when weighing their relative support.

(1) Parasites tend to be aggregated within hosts, most having a few parasites or none, whereas a few have many (Shaw and Dobson 1995; Shaw et al. 1998). As a result, the load distribution is right-skewed with a long tail, and empirically well described by the negative binomial distribution. An important implication of this pattern is that an accurate determination of parasite abundance in the host population requires a large sample size.

(2) Parasite distribution is expected to be heterogeneous over space. The spatial sampling in the current study covers the focal hybrid zone reasonably evenly, with sampling at 107 localities (Fig. 2), including about 30 scattered along the path of the zone center, each with close neighbors on either

side. This reduces the potential for a spurious match between the layout of those localities where hybrids are found and fluctuations in the spatial heterogeneity of parasites. Randomization of locality loads shows our sampling is unlikely to give false positive signals regarding hybrids. The locality sampling in the two previous studies was much reduced (12 localities across ≈150 km for Moulia et al. [1991], 22 localities across 200 km for Sage et al. [1986a]). The probabilities of false positives would therefore be higher, although the same test cannot be constructed for the available data. Of the six localities near the zone center where Sage et al. (1986b) found the 14 hyperinfected hybrid mice, three (15,16,18) occur within less than 10 km^2, two of these within 2.8 km of each other. Moreover, their dataset comprises an initial sampling and then a second sampling in a different season. Pinworms can display significant seasonal variations in prevalence and abundance in rodents (Haukisalmi et al. 1988; Abu-Madi et al. 2000). We suggest the combined effect of few localities, sequential sampling at two different seasons, and spatiotemporally heterogeneous parasites could lead to a false positive signal of pinworm load associated with hybrids in this dataset.

(3) Time lag between mouse trapping and dissection. In our study, the majority of mice were dissected the day after capture. In the study of Moulia and colleagues based only on pinworm infection, mice were kept two months in laboratory conditions before being dissected (Moulia et al. 1991). Pinworms are highly contagious in breeding facilities (Taffs 1976; Bazzano et al. 2002). Eggs in food, water, bedding, and airborne result in continual reexposure of mice to pinworms (Taffs 1976; Pritchett and Johnston 2002). We suggest this long lab-based time lag between capture and dissection may have severely influenced the pattern of pinworm infection reported by the authors.

These are the empirical reasons we do not feel the two reported patterns of hybrid parasitism should be treated as having equal support. We now turn to reasons arising from considering the evolution of host–parasite interactions.

MODELS AND EXPECTATIONS FOR HOST/PARASITE INTERACTION ON HOST SECONDARY CONTACT

While host taxa are isolated they diverge, but their parasites may diverge faster due to shorter generation time. If parasites reduce fitness significantly, then one part of the host genome must somehow keep up with this faster parasite evolution: the part controlling host immune response. Recombination is one way in which a set of host genes can quickly produce new combinations of alleles as yet unseen by contemporary parasites. This simple Red Queen, recombination argument has a number of notable implications.

(1) If recombination is the important source of new immune gene combinations, then coadaptation across those loci is unlikely—coadaptation requires combinations to be held together long enough for natural selection to act. This is unlikely if recombination is continually reshuffling them.
(2) On secondary contact, recombination between host immune genes from each source should produce rare combinations, thus allowing hybrids to avoid parasites targeting common genotypes.
(3) Vicariant sister taxa need not show high divergence for immune related genes—the Red Queen can be run by producing new (re)combinations of existing alleles, rather than fixing new mutations.

These implications are notable because the first argues against the existence of "coadapted gene complexes," the breakup of which would explain hybrid susceptibility with respect to parasite load (Sage et al. 1986a; Moulia et al. 1991). The second is consistent with the results of the current study, and argues against the hybrid unfitness due to parasite interactions proposed by those two studies. The third is consistent with evidence from genes implicated in the immune response. These often show trans-species polymorphism, for example the immunoglobulin heavy chain variable region (IgVH) (Su and Nei 1999; Esteves et al. 2005), the proteasome subunit β-type 8 (PSMB8) (Nonaka et al. 2000; Miura et al. 2010), the major histocompatibility complex (MHC) in general (Klein 1987) and across the two hybridizing mouse subspecies in particular (Čížková et al. 2011). Trans-species polymorphism is quite the opposite of high divergence in sister taxa (point 3 above).

Returning to the first of these three issues: we have suggested coadaption among immune genes is unlikely if they are using recombination to run a Red Queen race against parasites. Even if recombination is not breaking up coadapted gene complexes (Derothe et al. 2004), there is still reason to expect hybridization to produce suboptimal genotypes. This is because hybridization involves admixture of alleles that have never coexisted in the same population. In a single panmictic population, a new allele that is incompatible with a common allele is unlikely to increase in frequency, because it will mostly occur in individuals with the incompatible common resident. When a population is split in two, this filtering out of new incompatible alleles continues within each subunit but, as Dobzhansky (1936) and Muller (1942) pointed out, pairs of new alleles arising across the two isolated populations are, by definition, never tested against each other by natural selection. Secondary contact between the populations is the first time such pairs come together. During isolation, these alleles may have arisen to high frequency, and in this way unfit

(unfiltered) DMIs can be revealed at secondary contact, without either isolated subunit having had reduced fitness. Although the alleles concerned are usually thought of as being at different loci, the logic works similarly for alleles at the same locus, and the outcome is the same: admixture within/across loci is expected to reveal previously unseen (untested) incompatibilities. Here then is a clear potential cause of hybrid susceptibility that does not rely on coadaptation across sets of genes with high recombination. A further question then arises: why is all the evidence in the current study consistent with an improvement in hybrid immune response? In other words, why do we see no evidence of DMIs affecting host immune genes?

IS THE IMMUNE SYSTEM IMMUNE TO DMIs? TRANSITIVITY OF ALLELE COMPATIBILITY

Equality (=) is a transitive binary relation: if A = B and A = C, then B = C. Suppose allele compatibility (♥) was also a transitive binary relation: if A♥B and A♥C, then B♥C. The Dobzhansky–Muller model breaks this rule: if B and C are a pair of new alleles that arise across isolated subpopulations, while A denotes their original partner maintained in each vicar during subdivision, when B and C are brought together for the first time on secondary contact, transitive allele compatibility would imply they be compatible (because both are guaranteed compatible with allele A), rather than forming a DMI. This is one reason to favor the existence of DMIs between new alleles at different loci, rather than at the same locus: the constraints imposed by the common function of alleles at the same locus (encoding the same gene) make it more difficult to imagine how a new allele (B) could be incompatible with some alternatives (C), while compatible with others (A). Is there reason to suspect that allele compatibility should be strongly transitive for immune genes? If so, it would make immune gene DMIs unlikely. One circumstance favoring strongly transitive compatibility is in a system where many alleles are already being maintained. This is certainly true of the MHC (Klein 1986; Piertney and Oliver 2006). Then, to increase in frequency, a new allele must be compatible with very many partners. For N maintained alleles, the above transitive rule becomes:

If $\{A_1 \heartsuit B, A_2 \heartsuit B, \ldots, A_{N-1} \heartsuit B, A_N \heartsuit B\}$,
and $\{A_1 \heartsuit C, A_2 \heartsuit C, \ldots, A_{N-1} \heartsuit C, A_N \heartsuit C\}$, then B♥C.

The greater compatibility constraints on B and C with respect to overlapping sets of partners have two implications: in each vicariant population, new alleles are less likely to reach high frequency, and, if they do, they are more likely to be cocompatible on secondary contact. The first implication would lead to low turnover in common alleles during vicariance, and therefore trans-isolate (species) polymorphism. The second suggests, where private alleles do arise, they will be unlikely to form DMIs on secondary contact. That is, "systems where many alleles are already maintained are resistant to the development of DMIs during vicariance." This vicariant balancing selection scenario forms a natural counterpoint to Orr's argument regarding the preferential accumulation of DMIs in vicariant epistatic systems (Orr 1995; Kondrashov 2003).

The question of whether immune genes are immune to DMIs is immediately testable for the house mouse system: a survey of natural variation at two MHC loci has already been carried out in the two taxa (Čížková et al. 2011), a necessary prerequisite to examining MHC gene flow across the same region of the HMHZ for which, here, we have shown significantly reduced parasite load in hybrids. If the sharp distinction between the mouse taxa is maintained by DMIs at other loci, while immune genes are comparatively free of DMIs and introgressing alleles are favored, clear patterns should result. In the simplest case: wide clines at the MHC compared to other loci. In the more complex moving-hybrid-zone case suggested by Macholán et al. (2011): large geographic inclusions of introgressed MHC alleles behind the moving wave front. Work assessing these patterns is underway.

IS THERE STILL A ROLE FOR PARASITES IN HYBRID ZONES AND SPECIES BOUNDARIES?

Although the role of parasites in animal hybridization has been investigated over a wide range of models from invertebrates to mammals (Moulia 1999), no study has demonstrated without ambiguity that parasites perturb the outcome of hybridization. In the house mouse, our data show it is extremely unlikely that high helminth parasite load makes a significant contribution to the maintenance of the species boundary, contrary to what has been proposed over the past 25 years.

If there is a parasite influence on the HMHZ, our data would suggest it will be directional selection: with the exception of pinworms and male whipworms, *domesticus* mice are more heavily parasitized than *musculus* mice. Most strikingly, tapeworm load is eightfold lower on the *musculus* genetic background (Figs. 2E, 3D). Introgression of any responsible genes into the *domesticus* genome may therefore be favored. This may be related to a history of zone movement, *musculus* replacing *domesticus*, indicated by spatial X chromosome marker variation (Macholán et al. 2011). Mouse lab strains show heritable variation for tapeworm susceptibility (Olivier 1962; Orihara 1962). Larval tapeworms can strongly decrease fertility of both male and female rats (Lin et al. 1990). Excretory-secretory products of tapeworm larvae inhibit rat testosterone production (Rikihisa et al. 1985) and the tapeworm *T. crassiceps* can affect the seminiferous epithelium of male mice leading to infertility (Zepeda et al. 2011). In the Czech–Bavarian transect, the Y chromosome of *musculus* has introgressed into the territory of *domesticus*, covering more than 330 km^2 of Bavaria (Macholán et al. 2008). Resistance to a parasite capable of disrupting male reproductive function, such as the tapeworm, could facilitate this Y invasion, and dominant loci of major effect on

tapeworm resistance (*T. crassiceps*, Fragoso et al. 1996) would further aid the rapid spread of resistance, suggesting the role of tapeworms in the dynamics of the Czech–Bavarian hybrid zone is worth further investigation.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Simulating patches of low parasite occurrence falling at random on the field area.

Figure S1. Distribution of individual parasite diversity and load (blue histograms) across individuals, compared to expectations for the ML parameterization (red line) of the Poisson (A) and negative binomial (B–F) distributions assuming no difference between sexes and subspecies.

Figure S2. A least-squares fit of a two-level model for parasite diversity over radial distance from the centroid of the hybrids in the dataset.

Figure S3. An instance of a simulated patchy field area.

Figure S4. Contrasting likelihood analyze outcomes for patch-centered (left column) and patch-uncorrelated (right column) simulations. Table S1. Dataset per mouse individual.

Supporting Information may be found in the online version of this article.

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