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Research paper

Local adaptation of the MHC class IIβ gene in populations of wood frogs (*Lithobates sylvaticus*) correlates with proximity to agriculture

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ABSTRACT

Major histocompatibility complex (MHC) genes code for membrane-embedded proteins that are involved in parasite/pathogen recognition. The link between the MHC and immunity makes these genes important genetic markers to evaluate in systems where infectious disease is associated with population declines. As human impacts on wildlife populations continue to increase, it is also essential to evaluate the role of MHC and immunity in the context of anthropogenic change. Amphibians are an ideal model to test the role of the MHC in infectious disease resistance, as parasites and anthropogenic disturbances currently threaten populations worldwide. We characterized the diversity of MHC class IIβ peptide binding region alleles, 13 microsatellite loci, and population-level trematode resistance in 14 populations of wood frogs (*Lithobates sylvaticus*) in northwestern Pennsylvania with varying geographic distances to agriculture. To assess local adaptation in the MHC IIβ, we compared genetic differentiation of MHC IIβ and microsatellite markers (F_{ST}). We also tested for an effect of isolation by distance on genetic differentiation of MHC IIβ and microsatellite markers. In addition, we evaluated whether population-level MHC IIβ diversity and common allele frequencies correlate with distance to agriculture and trematode resistance. We found no evidence for genetic structure based on microsatellite analysis nor an effect of isolation by distance on neutral and immunogenetic markers. However, we did detect structure based on the MHC IIβ locus, suggesting that it is under local selection. The MHC IIβ allele Lisy-DAB*1 was more common in populations living near agricultural sites. Populations with higher MHC IIβ diversity showed increased resistance to trematodes. Our results suggest that wood frog populations experience immunogenetic differences at a small scale. In addition, agriculture may disturb natural associations between hosts and parasites through its influence on immunocompetence, underscoring the importance of examining the effects of environmental context on host-parasite interactions.

1. Introduction

The co-evolutionary arms race between hosts and parasites is a well-studied interaction across a diversity of taxa (Gandon et al., 2008). Pressure from parasites can result in changes to host immunogenotypes favoring disease resistance (Bernatchez and Landry, 2003). In contrast, parasites generally have smaller genomes, higher mutation rates, and shorter generation times than their hosts, which ensures that counter adaptation rapidly occurs in response to host defenses (Gandon and Michalakis, 2002). The major histocompatibility complex (MHC) is a useful genetic marker to track the adaptive responses of hosts to parasites (Bernatchez and Landry, 2003). For instance, parasite-mediated selection can favor heterozygosity in MHC loci (i.e. heterozygote advantage) or the maintenance of certain variants over generations through temporally fluctuating selection (i.e. negative frequency dependent hypothesis; O'Brien and Evermann, 1988). Thus, the arms race

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between hosts and parasites has propagated polymorphism in these genes across most taxa that possess them (Piertney and Oliver, 2006). Evaluating MHC diversity across populations can convey important information about the selective forces driving evolutionary processes between hosts and parasites.

To better understand the co-evolutionary interaction between hosts and parasites, there is a need to consider how external environmental conditions modify species assemblages. For instance, anthropogenic disturbances (e.g., agriculture) commonly influence ecosystems in ways that can alter host and parasite populations (Newbold et al., 2015). These disturbances can reduce biodiversity of hosts and parasites in terrestrial and aquatic environments (Aronson et al., 2014; Vallan, 2002; Lafferty, 2012), leading to homogenization of biotas and alteration of important ecological associations (Krauss et al., 2010). For example, several studies have documented increased infectious disease prevalence in areas experiencing biodiversity loss (Keesing et al., 2006; Ostfeld, 2009), suggesting that biota homogenization may favor certain parasites. Host populations that persist in or near environments altered by anthropogenic activities often adapt to these novel conditions. For example, in animals experiencing chronic exposure to pollutants, genetic responses in toxin metabolism and hormone-regulating genes are common (Williams and Oleksiak, 2008; Laporte et al., 2016). Given the evolutionary implications of anthropogenic disturbances on populations, it is important to examine how these evolutionary responses subsequently influence host-parasite interactions.

There is increasing evidence that agriculture can influence eco-evolutionary associations between hosts and parasites. High host densities and monocultures in agriculture can increase the probability of parasite spillover from domesticated to wild species (Lam and Chua, 2002). In addition, the global movement of domesticated species has caused a dispersal of associated parasites and an increased incidence of novel disease outbreaks in naïve wild hosts (Fevre et al., 2006). Land-use changes can also alter local parasite community richness, resulting in different parasite pressures between affected and unaffected habitats (King et al., 2010). As a consequence, agriculture-mediated shifts in parasite communities could influence the evolution of host populations. For example, both positive and negative correlations have been observed between MHC allele frequencies and a host population's proximity to agriculture activities (Gonzalez-Quevedo et al., 2016). These observations suggest that different parasite-exposure histories exist between environments with high- and low-agriculture disturbance, resulting in differential selection at local scales. Given the link between agriculture and parasite pressures, evaluating the landscape of immune-related traits in wild species across a gradient of anthropogenic disturbance is crucial to understand how human activities influence host-parasite interactions over time.

Amphibians are excellent models to evaluate evolutionary interactions between parasites, hosts, and agriculture. A broad diversity of parasites including fungi, viruses, and macroparasites negatively impact amphibian populations at a global scale (Kilpatrick et al., 2010; Daszak et al., 1999; Martel et al., 2013; Rajakaruna et al., 2008). In addition, biotic (e.g. presence of alternative hosts) and abiotic factors (e.g., temperature and hydroperiod) of habitats can contribute significantly to dynamics of amphibian infectious diseases (Blaustein et al., 2012). Environmental variation among habitats can influence the diversity of parasites that amphibian hosts are exposed to, parasite transmission potential, and host immune defenses (Orlofske et al., 2012; Martin et al., 2010). Further, fine-scale immunogenetic assessments of amphibian populations describe a genetic response to selective pressures caused by common pathogens/parasites. For example, both Teacher et al. (2009) and Savage and Zamudio (2011) identified directional selection in MHC peptide binding region (PBR) genes from populations experiencing ranavirus and chytrid fungus epidemics, respectively. Given the link between disease dynamics and environmental characteristics, observing a signature of immunogenetic response to habitat change is a possibility in amphibians.

Amphibian populations can vary in their response to habitat disturbance, dependent on population history of exposure to anthropogenic activities. Consistent with predictions of evolved pesticide tolerance, Hua et al. (2013, 2015) found that wood frog populations living close to agriculture possessed higher constitutive tolerance to carbaryl than those living far from agriculture. Interestingly, when individuals from the same populations were challenged with a trematode (*Echinoparyphium* sp.), populations living closer to agriculture were more resistant to infections than populations distant from agriculture (Hua et al., 2017). Collectively, these results suggest that the evolutionary mechanisms between resistance to parasite infection and anthropogenic pressures are related in this system. If so, we hypothesize that variation in exposure to agricultural pressures will influence patterns of local selection on immunogenetic traits.

We chose to focus our study on the partial MHC IIβ subunit 1 gene because this region is involved in antigen presentation from extracellular parasites (e.g. trematodes; Bernatchez and Landry, 2003). We tested whether selection or genetic drift influence MHC IIβ variation across the 14 populations studied in Hua et al. (2017). To ensure that the observed MHC IIβ patterns are driven by selection rather than by demographic processes (Pritchard et al., 2000a), we compared MHC IIβ structure to neutral genetic structure derived from 13 microsatellite loci. In addition, we evaluated whether isolation by distance correlates with neutral and MHC IIβ marker population differentiation. Given the short spatial distances between our populations (Fig. 1) and environmental heterogeneity, we expected MHC IIβ genetic structure to surpass neutral population-genetic structure due to differences in local-selection pressures. We also evaluated whether MHC IIβ diversity and common allele frequencies associate with a population's proximity to agricultural fields and whether patterns in MHC IIβ diversity and allele frequencies match population-level differences in resistance to *Echinoparyphium* sp. infection. In the context of our paper, we define resistance as the ability for the host to avoid getting infected. We expected a positive correlation between MHC IIβ diversity and distance to agriculture, as land-use change from agriculturally dominated to undisturbed areas has been associated with an increased diversity of parasite species (Lafferty, 1997; Bradley and Altizer, 2007; Brearley et al., 2013). Also, because of the role of the MHC IIβ in macroparasite defense, we predicted that the frequency of specific MHC IIβ alleles will correlate with population resistance to *Echinoparyphium* sp.

2. Material and methods

Specific details regarding the history of agriculture land-use near each population, tadpole husbandry, and experimental set up for tadpoles is described in previous papers (Hua et al., 2015, 2017; Table A.1). We measured a population's proximity to agriculture (defined as land used for crops, livestock, or game feedlots between 1993 and 2013) using Google Earth (2013, v. 7.1.2). We confirmed the agricultural status of proximate fields for each of our populations by visiting the fields, talking to landowners, and/or confirmation with local USDA agents (Hua et al., 2015). Next, we evaluated the susceptibility of each population to *Echinoparyphium* sp. by conducting an infection assay; the results of this assay are published in Hua et al., 2017. In short, on 8 April 2014, we collected 10 egg clutches from 14 populations in northwestern Pennsylvania (Fig. 1). A portion of each egg mass was shipped overnight on 21 April to the Purdue Wildlife Area (PWA) in West Lafayette, IN. The eggs for each population were placed into 100-L pools filled with 90 L of well water. On 28 April, all tadpoles had reached Gosner stage 25 and were fed rabbit chow ad libitum until the start of the experiments (Gosner, 1960). We exposed 15 tadpoles from each population to 50 *Echinoparyphium* cercariae obtained from infected snails that were collected at the PWA. We then quantified the trematode load (i.e. percentage of the 50 trematodes that encysted within each tadpole) via dissection three days post exposure. Previous studies have shown that this time frame is sufficient for parasites that

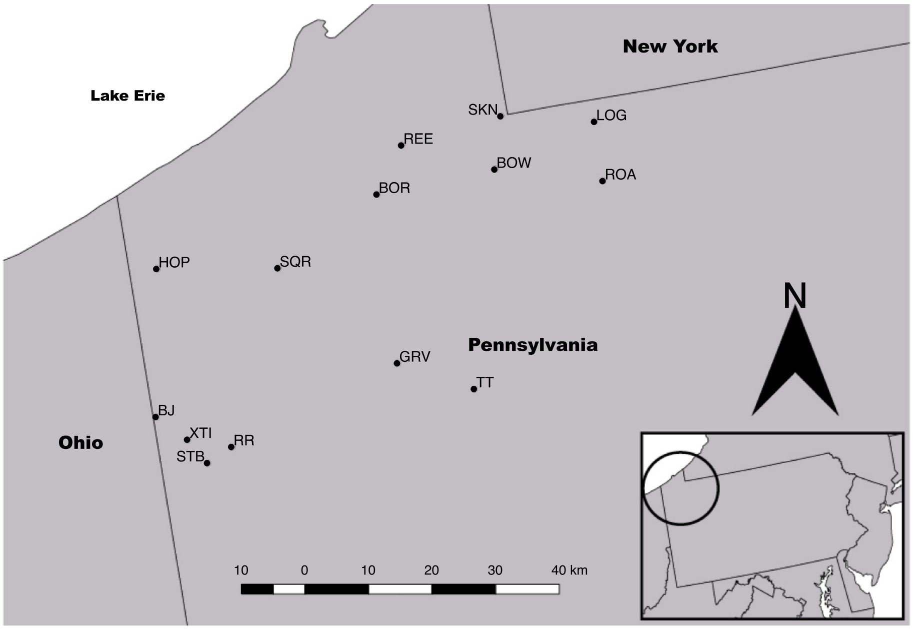

Fig. 1. Geographical locations of the 14 wood frog (*Lithobates sylvaticus*) populations used to characterize MHC IIβ, microsatellites, and resistance to parasites.

successfully infect the host to migrate into the kidneys and form cysts (Hua et al., 2017; Hoverman et al., 2013). Given that our hosts and parasites are from different geographic regions, local adaptation to this specific parasite population is unlikely. The tadpole carcasses where destroyed following dissection.

2.1. Genetic sample preparation

When tadpoles reached an average Gosner stage of 36.6 + 1.5 we randomly chose 25 tadpoles from each population for genetic analysis (although 30 individuals were chosen from population BOW) and euthanized them using an overdose of MS-222. The individuals were preserved and stored in 70% ethanol until DNA extraction. The tadpoles used for DNA extraction are not the same individuals used in the trematode challenge study. We obtained tail clips from each individual, and extracted DNA using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol, with the exception that we eluted the DNA with 50 μL of elution buffer instead of 200 μL.

2.2. Amplicon sequencing library preparation

We performed two sequential PCRs to amplify loci and insert TruSeq barcodes and Illumina sequencer adaptors to the ends of amplicons. The initial amplification was performed independently for each locus using primers derived from the literature (MHC IIβ peptide binding region and 13 nuclear microsatellites; Table A.2). However, we modified amplification primers with a linker sequence (forward primers – ACA CTCTTTCCCTACACGACGCTCTTCCGATCT; reverse primers – GTGACT GGAGTTCAGACGTGTGCTCTTCCGATCT) in the 5′ end that was used to attach barcodes and adaptors in the subsequent PCR. We amplified markers through 25 μL PCRs containing 7.5 ng/μL of template DNA, 0.4 μM of each primer (Table A.2), 1 U of Taq polymerase, 1 × reaction buffer (10 mM Tris-HCl, 50 mM KCl, 0.05 mg/mL BSA), 0.9 mM MgCl2, and 0.25 mM of each dNTP. We ran the reactions using the following thermal profile: 94 °C for 2 min, 94 °C for 30 s, primer specific annealing temperature (Table A.2) for 30 s, 72 °C for 30 s for 35 cycles, and a final elongation step of 72 °C for 10 min. We pooled 5 μL from each locus' PCR product by individual and cleaned each individual's amplicon pool using the UltraClean® PCR Clean-Up kit (MoBio Laboratories, Carlsbad, CA) following manufacturer's protocol.

The second PCRs were of 25 μL consisting of 5 μL of cleaned PCR product, 12.5 μL of MyTaq™ MasterMix (BioLine USA Inc., Tauton, MA), 0.4 μM of forward and reverse TruSeq barcode primers (Hernández-Gómez et al., 2017), and 6.5 μL of water. The barcoding PCR thermal profile consisted of 95 °C for 2 min, 5 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min. We quantified barcode PCR products using a Qubit Fluorometer (Invitrogen, NY) and pooled all samples in equimolar amounts. We cleaned the pooled samples using the UltraClean PCR Clean-Up kit following manufacturer's protocol. We submitted the cleaned samples to the Purdue Genomics Core Facility to be sequenced in one run on a MiSeq machine (Illumina, Inc., CA) using the V2 reagent kit to produce 250 bp paired-end reads.

2.3. Bioinformatics

We processed raw reads using Trimmomatic (Bolger et al., 2014) to remove adapter sequences, bases below threshold quality of phred-20 from both ends of reads, and any resulting reads under 30 bp. We paired reads that passed initial quality control using PANDAseq (Masella et al., 2012); only reads that paired successfully were used in subsequent analyses. We used a custom Python program (Appendix A) to remove quality scores from reads and separate reads by loci through 100% forward primer matching. Sequence data were uploaded to the NCBI Sequence Read Archive (https://ncbi.nlm.nih.gov/sra) under the Bioproject accession number PRJNA476003.

2.3.1. Microsatellite genotyping and structure analysis

We genotyped microsatellite loci by using a protocol similar to the Degree of Change criterion method described in Lighten et al. (2014). In short, we used a custom Python program (Appendix A) to create a list of all the unique sequences (variants) recovered for each individual, estimate the sequencing depth per variant (C_i), and rank the variants based on their sequence depth. We then calculated the rate of change (ROC_i) for each variant, which is defined as the difference in sequencing depth between each variant. We measured the proportion of depth change between each pair of variants (ROC_i/ROC_j) compared to the total amplicon depth for each marker (degree of change-DOC_i) for the first ten variants with the largest amplicon depth. The variant with the highest DOC value was determined as the first pseudo-allele, as this is the discriminant point where highest sequencing depth drop occurs. In our analyses, the discriminant point always occurred either at the second allele variant (corresponding to homozygosity in the locus) or the third allele variant (corresponding to heterozygosity in the locus). We discarded all pseudo-allele variants and named each microsatellite true allele based on its base pair length.

Individuals missing data from more than four loci were discarded. Microsatellite analyses were carried out in the R package POPGENREPORT (Adamack and Gruber, 2014) including tests for allelic richness, expected and observed heterozygosity, and estimate null alleles using the Chakraborty et al. (1992) method. To determine the number and membership of neutral genetic populations, we used a Bayesian clustering approach with MCMC estimation that randomly shuffles genotypes among candidate populations in order to minimize deviations from Hardy-Weinberg and linkage equilibria. We ran STRUCTURE (ver 2.3.2; Pritchard et al., 2000b) ten times each for all values of k from one to 14. We employed the correlated and admixture models because structure was expected to be weak at this geographic scale (Latch et al., 2011). We ran STRUCTURE without spatial priors for 100,000 burn-in iterations and 500,000 iterations thereafter. To determine the correct estimate of k from the output, we used a combined approach consisting of the Δk method (Evanno et al., 2005) as implemented in STRUCTURE HARVESTER (Earl and Vonholdt, 2012), the posterior probability of each k (Pr(X|K); Pritchard et al., 2000b), and STRUCTURE bar plots. To visualize consensus bar plots for each value of k across the ten replicates, we used the run averaging in CLUMPP (Jakobsson and Rosenberg, 2007) to produce barplots via DISTRUCT (Rosenberg, 2004) via CLUMPAK online (Kopelman et al., 2015).

2.3.2. MHC genotyping and diversity analysis

To discern between true MHC IIβ alleles and sequencing artifacts, we uploaded an un-multiplexed MHC IIβ amplicon read file to AmpliSAS (Sebastian et al., 2016). AmpliSAS is a web browser interface that performs read de-multiplexing, variant clustering, and putative allele filtering based on user specified criteria. We implemented AmpliSAS's default settings to remove any samples with < 100 read-depth and set the max number of alleles per amplicon to two given that only one copy of the MHC IIβ occurs in wood frogs (Kiemnec-Tyburczy et al., 2010). In addition, we directed AmpliSAS to filter out chimeric sequences and discard any variants within each individual if sequencing depth fell below a depth of 3.0%. This threshold excludes erroneous variants that do not cluster along with parental alleles (e.g., un-removed chimeras, contaminant reads, errors beyond the clustering algorithm). We BLASTed all variants designated as true alleles against the NCBI GenBank nucleotide database (Benson et al., 2018). We only retained variants that matched to the MHC class IIβ of ranids or other closely related amphibian species at an E-value < 0.05.

We aligned the MHC IIβ allele sequences with ClustalW (Larkin et al., 2007). We derived Tajima's D to evaluate deviations from neutrality in the MHC IIβ using MEGA 7.0 (Tajima, 1989; Kumar et al., 2016). A positive Tajima's D value indicates evidence for balancing selection or a recent population bottleneck, a negative value suggests the presence of selective sweeps or recent population expansions, and a value of zero corresponds with no evidence of selection.

To evaluate MHC IIβ diversity in each population, we computed the average percent difference (APD) per population as described in Yuhki and O'Brien (1990). APD corresponds to the average percentage of alleles that differed among individuals in each population. We then identified the best nucleotide substitution model for our MHC IIβ alignment using Bayesian Information Criteria in jModeltest v. 2.1.10 (Posada, 2008). We used the best-fit model (JC + I + G) in a maximum likelihood tree analysis in MEGA7 to visualize the relatedness of the MHC IIβ alleles (Kumar et al., 2016). Node support was estimated from 1000 bootstrap replicates. The 29 MHC IIβ allele sequences were deposited to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers MH481141-MH481169.

2.4. Statistical analysis

To evaluate if a correlation between neutral and MHC IIβ loci differentiation exists, we compared pairwise F_{ST} values calculated for each data set using a Mantel test. To investigate whether isolation by distance is at play, we also applied Mantel tests to compare pairwise population geographic distances and pairwise neutral and MHC IIβ F_{ST} values. We independently tested linear relationships between our dependent variables of population MHC IIβ characteristics (i.e. APD and relative abundance of alleles present in $> 10\%$ of individuals; Lisy-DAB*1 and Lisy-DAB*2) and log-transformed geographic distance to agriculture.

We also tested the effect of MHC IIβ characteristics on population-level resistance to trematode infection. For each population, we averaged the percent of trematodes that encysted across all individuals and used this value as our estimate of population-level trematode loads (Hua et al., 2017). Multivariate regression analysis was performed to evaluate the influence of population MHC IIβ characteristics (APD, Lisy-DAB*1, Lisy-DAB*2) on trematode loads. We compared models using the Akaike's information criterion (AIC) and selected a final model using backwards selection (Burnham and Anderson, 2002). To confirm the validity of the final model, we added discarded variables individually and checked whether this action improved model fit. The significance of the final model was assessed with the Wald test statistic.

3. Results

MiSeq sequencing resulted in 23,253,118 raw sequence reads. After merging and quality filtering, we used 8,473,310 reads in subsequent analyses. Sequencing depth for each individual locus varied from 300,431 to 1,014,568 reads, with a range of 848 to 2866 reads per individual (Table A.3). For each locus, we discarded samples whose sequencing depth was too low to confidently genotype (e.g. < 100 read depth). The percentage of individuals discarded per locus due to low sequence depth varied between 0.57% to 36.00% (Table A.3).

3.1. Microsatellite analysis

Thirteen individuals were discarded for having missing data at four microsatellite loci, leaving 337 individuals for downstream analyses (mean 24.0 per population; at least 22 individuals for all populations). The number of alleles per microsatellite locus ranged from 12 to 48 (mean: 25.1; Table A.4). The mean expected and observed heterozygosities were 0.878 and 0.601, respectively. There was some evidence for null alleles (Table A.4), but the exclusion of possibly problematic alleles did not substantially affect estimates of k. The Δk method results suggested $k = 4$ (Fig. A.1), but this method cannot exclude $k = 1$ as a better solution and is of relatively low magnitude (Janes et al., 2017). The Pr(X|K) method can be used to determine the optimal value of k at the point where it peaks or plateaus (Pritchard and Wen, 2003) but this method was inconclusive as the point estimate continued to rise from $k = 1$ to $k = 15$ (Fig. A.1). The bar plots did not support clear structure at any value of k (Fig. A.1). We therefore accepted $k = 1$ as the optimal value.

3.2. MHC IIβ genotyping

We characterized 29 MHC IIβ alleles with up to two MHC IIβ PBR alleles per individual, corresponding with previous observations of the MHC IIβ PBR in wood frogs (Kiemnec-Tyburczy et al., 2010). All 29 allele variants BLASTed to ranid MHC IIβ PBR. Our MHC IIβ Tajima's D value (1.47) was positive, indicating that balancing selection maintains diversity in the MHC IIβ loci across all populations sampled. In addition, we described variation in the diversity and frequency of alleles

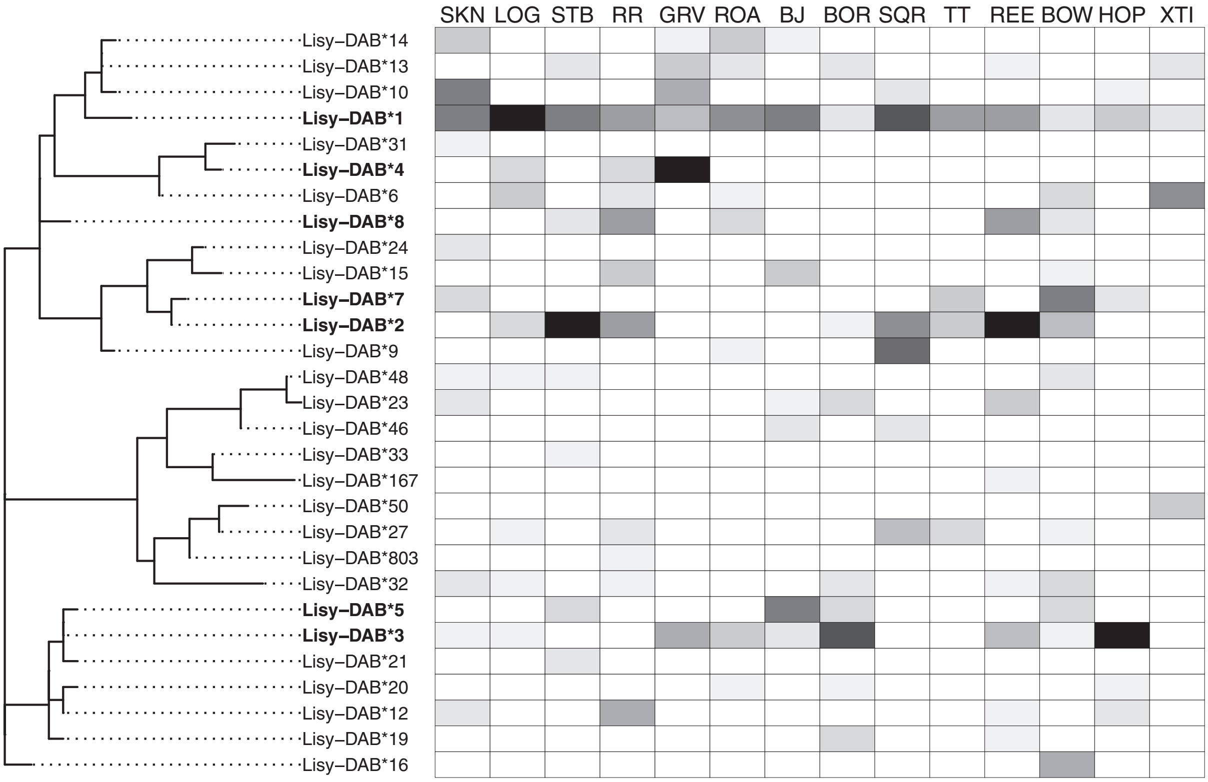

Fig. 2. Bootstrap consensus ML genealogy of 29 wood frog MHC IIB alleles. Heat map of allele relative abundances (0–0.6) in 14 wood frog populations from northwestern Pennsylvania is presented. Populations are ordered in increasing distance to agriculture from left to right. Common alleles (present in > 5% of individuals) are labeled in bold.

throughout all 14 populations (Fig. 2). Among the seven alleles present in > 5% of individuals (i.e. Lisy-DAB*1, Lisy-DAB*2, Lisy-DAB*3, Lisy-DAB*4, Lisy-DAB*5, Lisy-DAB*7, Lisy-DAB*8), we only observed phylogenetic clustering in two allele pairs: Lisy-DAB*2/Lisy-DAB*7 and Lisy-DAB*3/Lisy-DAB*5 (Fig. 2). Phylogenetic divergence among most common alleles suggests differences in functionality. Allele Lisy-DAB*1 was ubiquitous; however, its frequency varied from rare to dominant across all populations (Fig. 2). Lisy-DAB*2, Lisy-DAB*3, and Lisy-DAB*4, Lisy-DAB*5, Lisy-DAB*7, and Lisy-DAB*8 were common in some populations, but absent or rare in others (Fig. 2).

3.3. *Correlations between MHC IIβ and microsatellite patterns*

Pairwise F_{ST} values for microsatellite and MHC loci did not correlate ($R = 0.27$, $p = .104$), and MHC F_{ST} values varied between populations while microsatellite F_{ST} values remained fairly low across all comparisons (Table 1). We did not detect significant correlations between population geographic distances and neutral ($R = -0.13$, $p = .901$) or MHC ($R = -0.05$, $p = .683$) F_{ST} values. Together, these results suggest that our 14 populations constitute a single population with respect to neutral loci, but varying local selective forces (e.g. pressure from local pathogens) drive frequencies of MHC IIβ alleles to differ among all 14 populations.

3.4. *Correlations between MHC IIβ and population characteristics*

When evaluating the effect of distance to agriculture on the MHC IIβ locus, we did not observe significant linear relationships with APD ($F_{1,12} = 0.02$, $p = .903$) or the relative abundance of allele Lisy-DAB*2 ($F_{1,12} = 0.24$, $p = .636$). However, we noted a significant negative relationship between distance to agriculture and the relative abundance of allele Lisy-DAB*1 ($F_{1,12} = 5.49$, $p = .037$, estimate $= -0.063 \pm 0.027$; Fig. 3A). We detected parasites in all tadpoles used in the challenge assays. The final regression model comparing population MHC IIβ characteristics and trematode load found only APD to be a significant predictor (Table A.5). Tadpoles from populations with higher APD values experienced on average lower trematode loads (Fig. 3B, Table A.6, $p = .036$).

4. Discussion

We evaluated patterns of neutral and MHC IIβ genetic variation across 14 populations of wood frogs in northwestern Pennsylvania. These wood frog populations are known to vary in their proximity to agricultural fields, tolerance to carbaryl, and resistance to the trematode *Echinoparyphium* sp. (Hua et al., 2017). We noted no genetic structure based on microsatellite analysis; however, we did detect structure based on the MHC IIβ locus suggesting that it is under local selection among the 14 populations. We did not detect evidence of isolation by distance using microsatellite or MHC IIβ differentiation. When we evaluated the effect of distance to agriculture on population-level MHC IIβ characteristics, we observed that populations living closer to agricultural fields possessed higher relative frequencies of the Lisy-DAB*1 allele. We observed a negative relationship between MHC IIβ diversity (i.e. APD) and population-level trematode load.

4.1. *Microsatellite vs. MHC IIβ genetic variation*

We evaluated MHC IIβ differentiation against the background of neutral genetic variation. Neutral marker analysis revealed that the 14 wood frog populations are panmictic, indicating that gene flow occurs between the populations at rates high enough to largely negate genetic drift. This pattern is supported by the absence of isolation by distance on microsatellite divergence. Gene flow between the 14 populations is expected given the relatively small geographical scale of this study compared to the wood frog's extensive range throughout the United

Table 1
Pairwise F_{ST} values between wood frog (*Lithobates sylvaticus*) populations in northwestern Pennsylvania. Microsatellite loci derived F_{ST} values are below the diagonal line and major histocompatibility complex IIβ (MHC IIβ) values are above. Shading varies based on F_{ST} value with 0-white and 0.5 –black. F_{ST} values with a $p < .05$ are bolded. There are noticeable patterns of differentiation between populations based on MHC IIβ derived values compared to microsatellites.

	BJ	**BOR**	**BOW**	**GRV**	**HOP**	**LOG**	**REE**	**ROA**	**RR**	**SKN**	**SQR**	**STB**	**TT**	**XTI**
BJ		**0.08**	**0.07**	**0.12**	**0.17**	**0.08**	**0.08**	**0.06**	**0.06**	**0.06**	**0.08**	**0.09**	**0.07**	**0.14**
BOR	**0.04**		**0.07**	**0.10**	**0.05**	**0.14**	**0.06**	**0.06**	**0.08**	**0.08**	**0.10**	**0.12**	**0.11**	**0.14**
BOW	**0.04**	**0.04**		**0.11**	**0.15**	**0.11**	0.05	**0.07**	0.04	**0.06**	**0.07**	**0.07**	0.04	**0.09**
GRV	**0.03**	**0.04**	**0.04**		**0.16**	**0.13**	**0.11**	**0.09**	**0.09**	**0.08**	**0.12**	**0.14**	**0.11**	**0.14**
HOP	**0.03**	**0.03**	**0.03**	**0.03**		**0.22**	**0.14**	**0.12**	**0.16**	**0.15**	**0.18**	**0.22**	**0.19**	**0.24**
LOG	**0.03**	**0.03**	**0.04**	**0.04**	**0.03**		**0.10**	**0.06**	**0.08**	**0.07**	**0.07**	**0.09**	**0.05**	**0.14**
REE	**0.03**	**0.04**	**0.03**	**0.04**	**0.04**	**0.04**		0.05	0.03	**0.07**	**0.05**	0.02	0.05	**0.11**
ROA	**0.04**	**0.03**	**0.03**	**0.04**	**0.03**	**0.03**	**0.04**		0.04	0.04	0.05	**0.08**	**0.06**	**0.11**
RR	**0.04**	**0.03**	**0.03**	**0.04**	**0.03**	**0.03**	**0.04**	**0.03**		0.05	0.05	0.05	0.04	**0.09**
SKN	**0.04**	**0.03**	**0.03**	**0.04**	**0.03**	**0.03**	**0.03**	**0.04**	**0.03**		**0.06**	**0.09**	0.05	**0.11**
SQR	**0.04**	**0.04**	**0.04**	**0.05**	**0.03**	**0.03**	**0.04**	**0.03**	**0.03**	**0.04**		0.05	0.04	**0.12**
STB	**0.04**	**0.04**	**0.04**	**0.05**	**0.04**	**0.03**	**0.04**	**0.04**	**0.04**	**0.03**	**0.03**		0.05	**0.15**
TT	**0.04**	**0.04**	**0.04**	**0.04**	**0.03**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**		**0.14**
XTI	**0.05**	**0.05**	**0.05**	**0.06**	**0.04**	**0.04**	**0.05**	**0.04**	**0.05**	**0.04**	**0.04**	**0.04**	**0.05**	

States and Canada, and the documented evidence of long-range migrations in this species (> 1 km; Bellis, 1965, Berven and Grudzien, 1990, Baldwin et al., 2006). However, despite the absence of neutral genetic structure, we noted differentiation of populations based on the adaptive MHC IIβ loci independent of inter-population geographic distance. Discordance between MHC and neutral loci suggests that spatial environmental variation can serve as a mechanism that shapes MHC variation (Morjan and Rieseberg, 2004). In fact, similar patterns have been documented in other animal systems, where inter-population variation in the MHC contrasts with variation observed in neutral markers (Alcaide et al., 2008; Sallaberry-Pincheira et al., 2016; Kosch et al., 2016). Differences in environmental conditions and population histories (e.g. latitude, habitat quality, disease history, mating histories, bottlenecks) can drive variation in MHC structure among populations (Sallaberry-Pincheira et al., 2016; Radwan et al., 2014; Talarico et al., 2019; Cortázar-Chinarro et al., 2017). However, the absence of population variation and allele drop-offs in the adaptive and neutral loci suggest that only selection history is driving the patterns observed in the current study and not other demographic processes such as founder effects (Nei et al., 1975). Therefore, our MHC IIβ versus microsatellite comparisons suggest that the wood frog MHC IIβ experiences local selection across the environmental gradient.

4.2. *MHC IIβ and population distance to agriculture*

We found an association between the frequency of one common MHC IIβ allele and distance to agriculture, indicating that selection on the MHC IIβ can vary along this environmental gradient. While a population's distance to agriculture does not entirely provide information related to the environmental conditions experienced by amphibians (e.g., history of chemical use at a site, topography, canopy cover, water depth), unmeasured environmental factors could also be of importance to patterns of local selection in our populations. In the context of our study, agricultural activities could affect the diversity and prevalence of parasites in wood frog-inhabited ponds. Inter-population variation in allele frequencies can occur in response to differences in local disease patterns (Sutton et al., 2011). Within amphibian-disease systems, human activities can modify disease prevalence, but these effects can be highly variable (Brearley et al., 2013). For example, a reduction in amphibian parasite diversity has been noted in wetlands impacted by

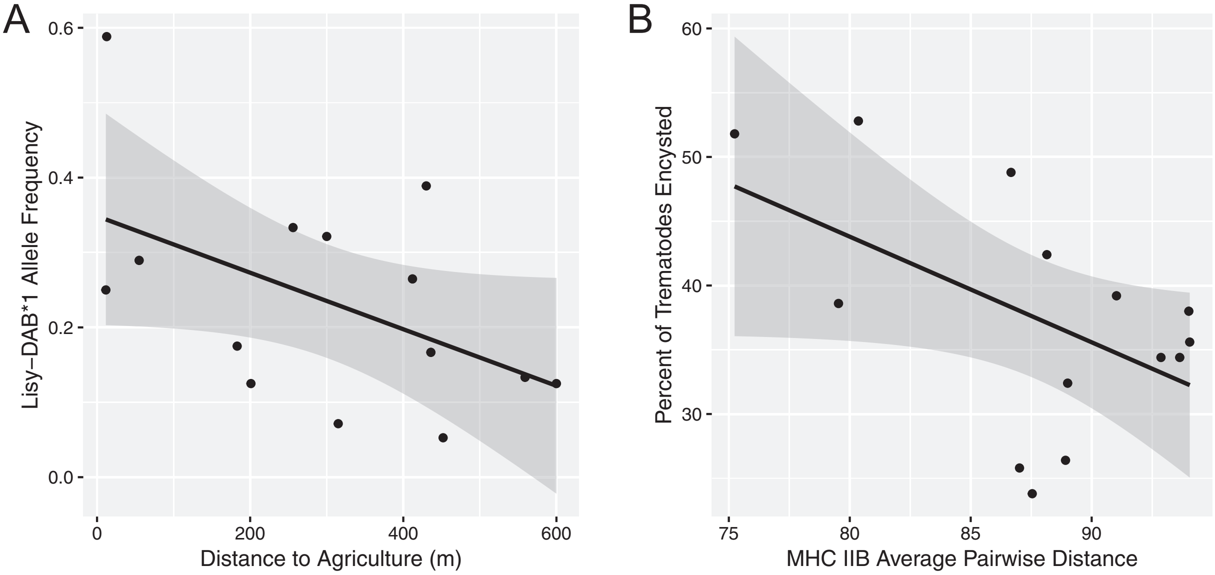

Fig. 3. Linear relationships between (A) log-transformed distance to agriculture and the relative abundance of MHC IIβ allele Lisy-DAB*01, (B) MHC IIβ average pairwise distances and average trematode load wood frog tadpoles from 14 populations in northwestern Pennsylvania. Shading corresponds to the 95% confidence interval for predictions from the linear models.

agricultural land-use compared to those in natural habitats (King et al., 2010). However, parasites can respond differently to agriculture pressures creating a complex gradient of selection. Both King et al. (2010) and McKenzie (2007) describe the proliferation of distinct parasites in natural vs. agriculture dominated habitats. Because parasite-mediated selection influences MHC evolution (Piertney and Oliver, 2006), the wood frog populations may differ in their history of exposure to relevant parasites that were not measured in this study (e.g. *Batrachochytrium dendrobatidis*, ranaviruses, bacteria) driving differences in the frequency of Lisy-DAB*1 along an agricultural gradient. As a result, future work should evaluate the prevalence and infection intensity of amphibian pathogens/parasites across natural and human-altered landscapes, and assess correlations between parasite prevalence, environment, and immunogenetics.

4.3. MHC IIβ and resistance phenotype

The APD of the MHC IIβ locus in the studied wood frog populations correlated with the infection load of *Echinoparyphium* sp., indicating that diversity of the MHC IIβ is associated with population resistance to trematode infection. This finding is important given that amphibians with short tadpole stages, such as wood frogs, show increased susceptibility to echinostome infections (Johnson et al., 2012; Rohr et al., 2010). One limitation is that our study design did not allow us to compare individual MHC IIβ genotypes to individual parasite loads. This type of analysis has been implemented to describe associations between adaptive MHC IIβ alleles and resistance to amphibian pathogens (Savage and Zamudio, 2011). At the individual level, MHC diversity has been associated with resistance to pathogen infection (Kurtz et al., 2004; Hawley and Fleischer, 2012), as higher MHC diversity may allow more diverse peptides derived from pathogens to be detected and cleared (Kurtz et al., 2004). However, a link between MHC diversity and parasite resistance is also evident in population-wide studies, where populations possessing higher MHC allele diversity have been observed to possess lower parasite burdens (Meyer-Lutch and Sommer, 2009). One possible explanation for this finding is that populations with higher MHC diversity likely hold a greater number of individuals that possess protective MHC IIβ alleles. Given the associations between MHC IIβ diversity, MHC IIβ allele frequencies, and disease resistance, our results suggest that a population's resistance to a parasite challenge may depend on the population's history of exposure to human activities (e.g. agriculture) and its consequential effect on adaptive genetic diversity. Collectively, these data demonstrate the evolutionary implications of agricultural land use by demonstrating its effect on the future immunogenetic potential of wild amphibians.

5. Conclusions

Because of the current threat of disease to amphibian populations, an ongoing need is to investigate how anthropogenic environmental changes dynamics between parasites and hosts, as these could lead to increased pathogenicity and/or decreased susceptibility (Jimenez and Sommer, 2017). Our study addressed this need by evaluating the immunogenetics of wood frogs under a gradient of agriculture disturbance, and the response these interactions have on resistance to a common amphibian parasite. We show that variation in selection forces along an agricultural gradient can lead to different patterns of local selection on the MHC IIβ. Our results highlight the need to consider the effects of habitat quality on host adaptation when inferring susceptibility patterns in amphibians. Given that immunity in amphibians is a complex trait (Woodhams et al., 2014), more studies are necessary to assess the effects of human activities on other aspects of amphibian immunity. Because human activities can also influence multiple components of amphibian physiology (e.g. microbiome, innate immune system, adaptive immune system; Krynak et al., 2016, Becker et al., 2017, Hughey et al., 2017, Woodhams et al., 2014), human activity has the potential to influence multiple aspects of amphibian immunity. Thus, future research should continue to integrate host and environmental characteristics to accurately assess variation in host-parasite associations across populations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.04.032.

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