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ARTICLE

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Host plant-mediation of viral transmission and its consequences for a native butterfly

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Abstract

Pathogens play a key role in insect population dynamics, contributing to short-term fluctuations in abundance as well as long-term demographic trends. Two key factors that influence the effects of entomopathogens on herbivorous insect populations are modes of pathogen transmission and larval host plants. In this study, we examined tritrophic interactions between a sequestering specialist lepidopteran, *Euphydryas phaeton*, and a viral pathogen, Junonia coenia densovirus, on its native host plant, *Chelone glabra*, and a novel host plant, *Plantago lanceolata*, to explore whether host plant mediates viral transmission, survival, and viral loads. A two-factor factorial experiment was conducted in the laboratory with natal larval clusters randomly assigned to either the native or novel host plant and crossed with either uninoculated controls or viral inoculation (20% of individuals in the cluster inoculated). Diapausing clusters were overwintered in the laboratory and checked weekly for mortality. At the end of diapause, all surviving individuals were reared to adulthood to estimate survivorship. All individuals were screened to quantify viral loads, and estimate horizontal transmission postmortem. To test for vertical transmission, adults were mated, and the progeny were screened for viral presence. Within virus-treated groups, we found evidence for both horizontal and vertical transmission. Larval clusters reared on the native host plant had slightly higher horizontal transmission. Survival probability was lower in clusters feeding on the native host plant, with inoculated groups reared on the native host plant experiencing complete mortality. Viral loads did not differ by the host plant, although viral loads decreased with increased sequestration of secondary compounds on both host plants. Our results indicate that the use of a novel host plant may confer fitness benefits in terms of survival and reduced viral transmission when larvae feeding on it are infected with this pathogen, supporting hypotheses of potential evolutionary advantages of a host range expansion in the context of tritrophic interactions.

KEYWORDS
Chelone glabra, disease ecology, *Euphydryas phaeton*, host plant, insect pathogens, *Plantago lanceolata*, secondary chemistry, tritrophic interactions

INTRODUCTION

Insect pathogens have been studied primarily in the context of their applications for biocontrol in crop pest management (Hajek & Delalibera, 2010; Lacey et al., 2015; Wraight & Hajek, 2008), but their importance in population regulation in unmanaged systems is receiving increased attention (Cory & Myers, 2009; LeBrun et al., 2022; Myers & Cory, 2016). Population regulation by pathogens occurs through both lethal and sublethal effects; in other words, pathogens can directly influence populations through increased mortality, or by reducing host fitness through decreased net reproduction, or altered developmental rates (Bell & Kanavel, 1976; Rothman & Myers, 1996). A pathogen's effect on an insect population ultimately depends on the modes of transmission, the transmissibility, and virulence of the pathogen, the population size and density of the hosts, and the immunocompetence of the host. All of these are potentially mediated by their host plant species (Cory & Hoover, 2006; Smilanich et al., 2018).

Transmission of pathogens is key to population-level effects on insects and can occur horizontally, where the pathogen is passed from one infected individual to another, or through environmental contamination (indirect transmission) (Goertz et al., 2007; Hajek & Shapiro-Ilan, 2018). In contrast, some entomopathogens are spread through vertical transmission, or transmission from parent to offspring, demonstrated in certain viral lineages such as nucleopolyhedroviruses, which are commonly used as biocontrol agents (Vilaplana et al., 2010; Virto et al., 2014), and in densoviruses (Morais et al., 2020).

Research into the role of host plants in modulating the impact of pathogens on insects has expanded in recent decades (Cory & Hoover, 2006; Resnik & Smilanich, 2020; Shikano, 2017; Smilanich et al., 2018). Studies have typically focused on the immune response or other common performance measures, such as survival and growth rates, in response to intraspecific variation in host plant quality or different host plant species (Cory & Hoover, 2006; de Roode et al., 2008; Shikano et al., 2010; Smilanich et al., 2009). Novel, or recently colonized, plants are often viable hosts for insect herbivores, but tend to be associated with reduced performance relative to ancestral hosts (Forister et al., 2009; Muchoney et al., 2022; Yoon & Read, 2016). Secondary metabolites are a key component that may underlie these host plant-mediated differences (Harvey & Fortuna, 2012; Smilanich et al., 2018; Smilanich & Muchoney, 2022). In the context of tritrophic interactions, putatively toxic compounds can create "enemy-free" or "enemy-reduced space" in which insects exhibit greater resistance and tolerance to natural enemies such as pathogens, parasites, and predators by exploiting plant secondary chemistry via sequestration (Dyer & Bowers, 1996; Muchoney et al., 2022; Tan et al., 2019). For example, in Baltimore checkerspots (*Euphydryas phaeton*, Nymphalidae), sequestering high concentrations of iridoid glycosides resulted in lower viral loads (Muchoney et al., 2022). Similarly, monarch butterflies (*Danaus plexippus*) reared on diets with medium to high cardenolide concentrations showed reduced parasite load and growth (Gowler et al., 2015; Sternberg et al., 2012). Both examples highlight the importance of host plant chemistry and sequestration for defense against pathogens.

To investigate how host plant species affect the susceptibility to, and transmission of, a viral pathogen (Junonia coenia densovirus [JcDV]: *Parvoviridae*), we used a native, sequestering, North American lepidopteran, *E. phaeton* (Nymphalidae), that feeds on both a native and a novel host plant. Our objectives were to (1) investigate the influence of host plant consumed on horizontal and vertical viral transmission, (2) determine whether viral loads, survival, and fecundity are host plant dependent, and (3) quantify the effect of sequestration of iridoid glycosides found in the host plants on insect viral load. Based upon prior knowledge of the system (Muchoney et al., 2022), we predicted that the native host plant would serve as a higher quality food source, and thus would support higher resistance to the pathogen that would manifest in lower transmission and viral loads. Additionally, we predicted that high levels of chemical sequestration would reduce viral loads in surviving adult *E. phaeton*. Last, we predicted that survival and fecundity would be higher on the native host, and that viral inoculation would negatively impact these measures of fitness.

METHODS

Study system

The Baltimore checkerspot (*E. phaeton* Drury, Nymphalidae) is an oligophagous, univoltine nymphalid butterfly occurring in the eastern United States (Scholtens, 1990). Caterpillars undergo six larval instars, feed gregariously as prediapause larvae, and diapause with their sibling groups as fourth instar larvae (Bowers, 1980). Larval host plants of *E. phaeton* that co-occur in the northern part of the range include the native *Chelone glabra* (Plantaginaceae), or white turtlehead, and the introduced *Plantago lanceolata* (Plantaginaceae), commonly known as ribwort plantain or narrow-leaved plantain. The host range expansion of *E. phaeton* to include *P. lanceolata* as an oviposition plant was first described in the late 1970s (Stamp, 1979), and has since been the topic of several studies examining the consequences of this novel host shift (Bowers et al., 1992; Brown et al., 2017; Muchoney et al., 2022). *Euphydryas phaeton* larvae are chemical specialists on plants containing iridoid glycosides (IGs), which they sequester at relatively high concentrations and use as a defense against natural enemies, including both predators and pathogens (Bowers et al., 1992; Carper et al., 2022; Mason & Deane Bowers, 2017; Muchoney et al., 2022). *Chelone glabra* and *P. lanceolata* contain the same two IGs, aucubin and catalpol; however, the relative concentrations of these two compounds differ between the two species, with *C. glabra* containing high concentrations of catalpol, and sometimes aucubin in low concentrations, and *P. lanceolata* containing more aucubin or both compounds in relatively equal concentrations (Bowers et al., 1992, 1993; Bowers & Puttick, 1986).

Densoviruses are a group of single-stranded DNA viruses that are distributed across invertebrate taxa including Lepidoptera (Mutuel et al., 2010). The JcDV (*Parvoviridae*: *Densovirinae*: *Protoambidensovirus*: *Protoambidensovirus lepidopteran1*) is a small DNA virus that was originally isolated from the common buckeye butterfly (*Junonia coenia*: Nymphalidae) and has since been found in several other lepidopterans, including other nymphalids and *E. phaeton* (François et al., 2016; Muchoney et al., 2022; Mutuel et al., 2010; Rivers & Longworth, 1972). Infection by JcDV can occur through oral ingestion, after which it crosses the midgut, and replicates in the tracheae and hemocytes, and can cause mortality through hypoxia and molting arrest (Mutuel et al., 2010; Vendeville et al., 2009). The efficacy of horizontal transmission and the presence of vertical transmission of JcDV is undocumented in *E. phaeton*. Vertical transmission is possible as JcDV has been detected transovum (egg surface) in at least one other butterfly host (*Vanessa cardui*: Nymphalidae) (Smilanich et al., unpublished data), suggesting background infection can exist even within laboratory-reared populations. In other densoviruses, horizontal transmission can occur through excretion of infected cells or through cannibalism of infected insects (Miller et al., 1999), and vertical transmission is thought to occur in densoviruses that infect mosquitos (Altinli et al., 2019).

Experimental methods

The experimental configuration consisted of a fully crossed design for unique treatment-level combinations of the host plants (native vs. novel), and viral treatments (orally inoculated or uninoculated control) (Figure 1a). Given that females oviposit clusters of eggs, these clusters were kept intact and randomly assigned to treatments, placed into separate, sterilized, plastic containers, and provisioned with leaves from the assigned host plant upon hatching (1473 individuals from 25 clusters). Caterpillar clusters ranged in size from 7 to 143 individuals, with an average of 59 individuals per cluster. All host plant leaves were surface sterilized with a 5% bleach solution and rinsed prior to feeding. Once larvae reached the second instar, the "JcDV inoculated" groups were taken to a separate laboratory for viral inoculation and kept there for the remainder of the experiment (see Appendix S2 for additional details on sterile technique).

Horizontal transmission

In each "JcDV inoculated" cluster, 20% of individuals were randomly selected for viral inoculation, which is a field-relevant infection frequency for wild populations (Muchoney et al., 2022). These individuals were temporarily separated from their clusters and orally inoculated by pipetting the virus onto a leaf segment and feeding it to the selected subgroup (Figure 1b). Caterpillars were inoculated in batches by exposing them to viruses pipetted onto host plant material. One microliter of 1×10^6 viral equivalent genomes/microliter PBS suspension of JcDV per individual, multiplied by the number of individuals was pipetted using a 10-μL micropipette (Pipette ONE) onto a standardized leaf area (40 mm^2 per individual multiplied by the number of individuals). Purified virus stock was obtained from M. Ogliastro at the University of Montpellier, France. Caterpillars were then given 48 h to consume the leaf material, thus becoming infected with the virus (modified from Smilanich et al., 2018). Some mortality of individuals ($N = 29$) occurred during the period immediately after inoculation. After this period, the inoculated individuals were placed back into their natal clusters and reared with their siblings, before initiating diapause beginning in September 2020 (for details on the overwintering process see Appendix S2). Larvae were brought out of diapause beginning in May 2021. High mortality occurred

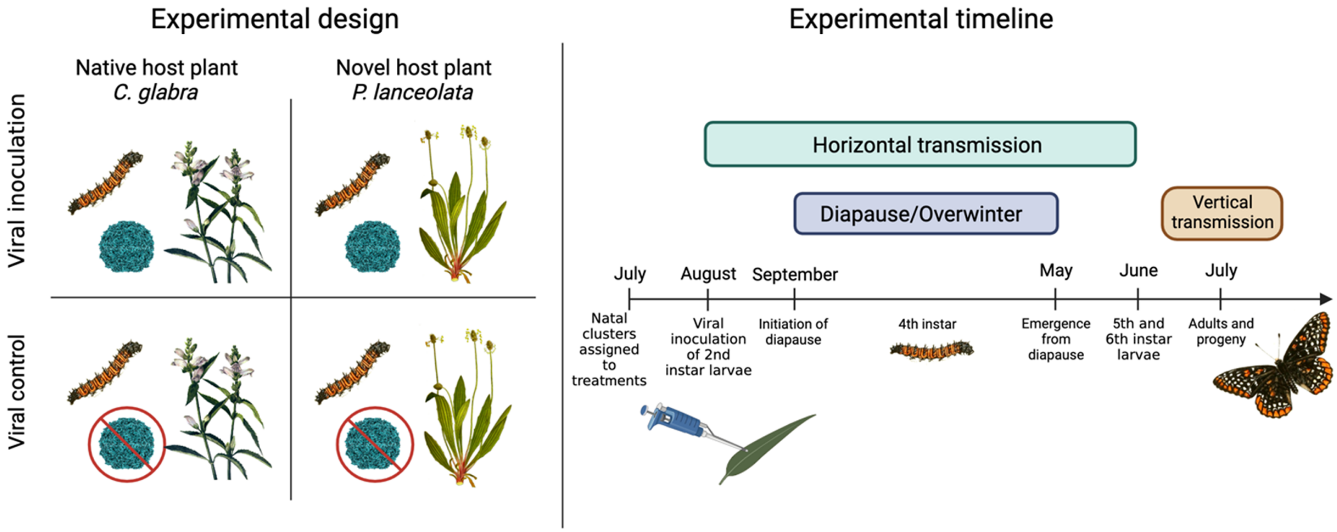

FIGURE 1 An overview of the experimental design (a) in which *Euphydryas phaeton* were assigned to either the native host plant (*C. glabra*) or the exotic host plant (*P. lanceolata*), and inoculation by Junonia coenia densovirus or a control group (no viral inoculation). The experimental timeline (b) documenting major experimental events starting in the summer of 2020. Egg clusters were assigned to treatment groups in July, and viral inoculation occurred in August while caterpillars were in the second instar. Caterpillars were placed into diapause over several weeks by lowering incubator temperatures and day/night cycles while they were in their fourth instar, and the reverse process was initiated in May to end the diapause. Pupation occurred over several weeks in June, and adults emerged in June and July. All individuals from the first generation were reared to natural mortality and screened for viral loads at all life stages from the fourth instar onward. Surviving adults were mated haphazardly in July, and second-generation offspring were reared to the third instar before they were sacrificed for viral screening. Brandon Collins created digital artwork of *E. phaeton*, *P. lanceolata*, and *C. glabra* from original illustrations by William Henry Edwards, Carl Axel Magnus Lindman, and Mary Vaux Walcott.

either during or just post diapause ($N = 816$). The first date of each life stage (i.e., instar, pupa, adult) observed for each cluster was recorded throughout the duration of the experiment to track phenology. Dead individuals were removed and recorded daily, and frozen for subsequent viral load analysis via qPCR. All individuals were reared to natural mortality in order to estimate survival through the lifespan.

Vertical transmission

To assess vertical transmission of JcDV, recently eclosed adults from the initial clusters (i.e., survivors from the horizontal transmission experiment) were placed together with siblings in mating cages outdoors in the summer of 2021. They were provisioned on honey water and monitored daily, and females with mating plugs as well as dead individuals were removed. Gravid females were placed singly into 15.7-in × 15.7-in × 24-in mesh oviposition chambers (Cambro) and monitored until they oviposited or died, at which point they were frozen for future viral screening. To maximize the probability of oviposition, all gravid females were placed in chambers with the native host, *C. glabra*, as it is the preferred oviposition plant of *E. phaeton* (Bowers et al., 1992; Bowers & Richardson, 2013). Hatched progeny were reared on the original host plant assigned to their parents until the third instar, then freeze killed. From this second generation, we sampled 50% of each natal cluster at the third instar for the detection of JcDV. For the parental generation, all individuals from the fourth instar onward were screened for viral loads of JcDV using qPCR including the control clusters (see Appendix S2 for viral detection protocol).

DATA ANALYSIS

All statistical analyses were performed using R (version 4.0.5). Bayesian generalized multilevel models were implemented using the R package *brms* (version 2.18.0; Bürkner, 2018). Wherever relevant information was available, weakly informative priors were generated from previously published results on the same study system (Muchoney et al., 2022). In Bayesian analysis, informative priors are incorporated into models by assigning probability distributions that represent possible parameter values based on previous knowledge of or data from the system (Lemoine & Lemoine, 2019; McElreath, 2018). Our models were run with both informative and uninformative priors, which had little effect on parameter estimates (see Appendix S1: Table S4 for full descriptions of priors used). Prior to statistical analysis, clusters that had two or fewer individuals that made it to the viral screening stage (4th instar or later) were omitted (five clusters) from models. Appendix S1: Table S1 shows a summary of the clusters included in the viral analyses. To estimate the effects of viral inoculation and host plant on horizontal transmission, we used a Bayesian generalized linear mixed model (GLMM) with a Bernoulli probability distribution and a logit-link function, with a variable intercept for cluster, and host plant and viral inoculation as fixed predictors. Horizontal transmission is defined as the probability of testing positive for JcDV at the time of death or the completion of the experiment. In addition to the model estimates, we also express it as the average final viral prevalence minus the initial infection frequency. To assess vertical transmission, we used a Bayesian GLMM with the viral status of the mother (0 = negative, 1 = positive) as the predictor, with a variable intercept term for the cluster, and the viral status of the offspring (0 = negative, 1 = positive) as the outcome variable. For all models, we used four Markov chains with 6000 iterations, and 500 burn-in steps. We assessed performance by visual examination of the chains as well as the Gelman and Rubin convergence diagnostic (Gelman & Rubin, 1992). Model comparison of several candidate models was conducted by assessing Watanabe Information Criteria (WAICs) (Watanabe, 2021).

Analyses for viral loads were conducted only with individuals from the first generation that met the criteria for testing “positive” for JcDV ($n = 390$), which included individuals from both the viral inoculation group and the control group. For estimating the effects of viral inoculation and host plant on viral loads, we used Bayesian linear mixed models (LMMs), with a normal error distribution and identity link with a variable intercept term for the cluster. The response variable was log-transformed relative viral loads (relative amount of viral DNA to total insect DNA). Fecundity was treated as the number of caterpillars from the second generation that hatched and survived to the third instar, as it was only possible to obtain accurate counts once caterpillars were larger and nonmobile. For all models, we used four Markov chains with 6000 iterations, and 500 burn-in steps. We assessed model performance as described above.

To assess the effects of experimental treatments on survival to adulthood, Bayesian GLMMs (i.e., logistic regression with treatment factors as fixed predictors and cluster as a variable intercept term) were utilized and model WAICs were compared. All individuals from the first generation that successfully hatched were used in this analysis ($N = 1473$). To examine survival to adulthood as a response to viral loads, a Bayesian logistic regression was conducted, and for viral loads as a function of life stage, a Bayesian linear regression was employed.

To evaluate the direct and indirect causal relationships between host plant and viral inoculation, and cluster density, on viral transmission, viral loads and survival we employed Structural Equation Modeling (SEM). Similarly, we used this approach to assess the effects of host plant and viral inoculation on chemical sequestration, and its impacts on viral loads in adults. A priori models were proposed based on the questions and predictions of the experiment and knowledge of our study system, and candidate models were statistically compared. Our approach used only observed variables (i.e., latent variables are not included). SEM decomposes total effects into direct and indirect (mediated) effects of multiple predictors on multiple outcomes (Garrido et al., 2022). Global estimation using the *lavaan* package (version 0.6–12) in R was employed (Rosseel, 2012). All predictor variables were converted to z-scores (see Appendix S2 for additional details on variables), and the model including viral loads only used data for individuals that tested positive for JcDV. Model goodness-of-fit was assessed using a chi-squared statistic, where higher p-values (i.e., $p > 0.05$) indicate the modeled covariance matrix is not significantly different from the observed covariance matrix (Lefcheck, 2016). Additionally, the Root Mean Square Error of Approximation, Comparative Fit Index, and Tucker–Lewis Index were considered (Marcoulides et al., 2019).

RESULTS

We found that both horizontal and vertical transmission of JcDV takes place in this system. There was a clear and strong effect of JcDV inoculation on the probability of contracting JcDV compared with the uninoculated control groups (odds ratio = 7.32, 95% Credible interval [CI] = [4.01, 13.60]), with substantial spread in inoculated clusters (28% growth in viral prevalence from initial inoculation frequency). The effect of the host plant on the average transmission was small but indicated that horizontal transmission was lower on the novel host plant (odds ratio = 0.89, 95% CI = [0.49, 1.56]); novel, control = 12% JcDV positive; native, control = 12% JcDV positive; novel, inoculated = 46% JcDV positive; native, inoculated = 54% JcDV positive, (Figure 2a). Within clusters reared on the native host plant, there was substantial variation in JcDV prevalence compared with those reared on the novel host plant (*C. glabra* range: 0.23–0.73; *P. lanceolata* range: 0.3–0.54; Figure 2b). For vertical transmission, individuals whose mothers tested positive for JcDV were more than six times as likely to test positive than those whose mothers were negative for JcDV (odds ratio = 6.62, 95% CI = [0.18, 254.96]) (Figure 2c).

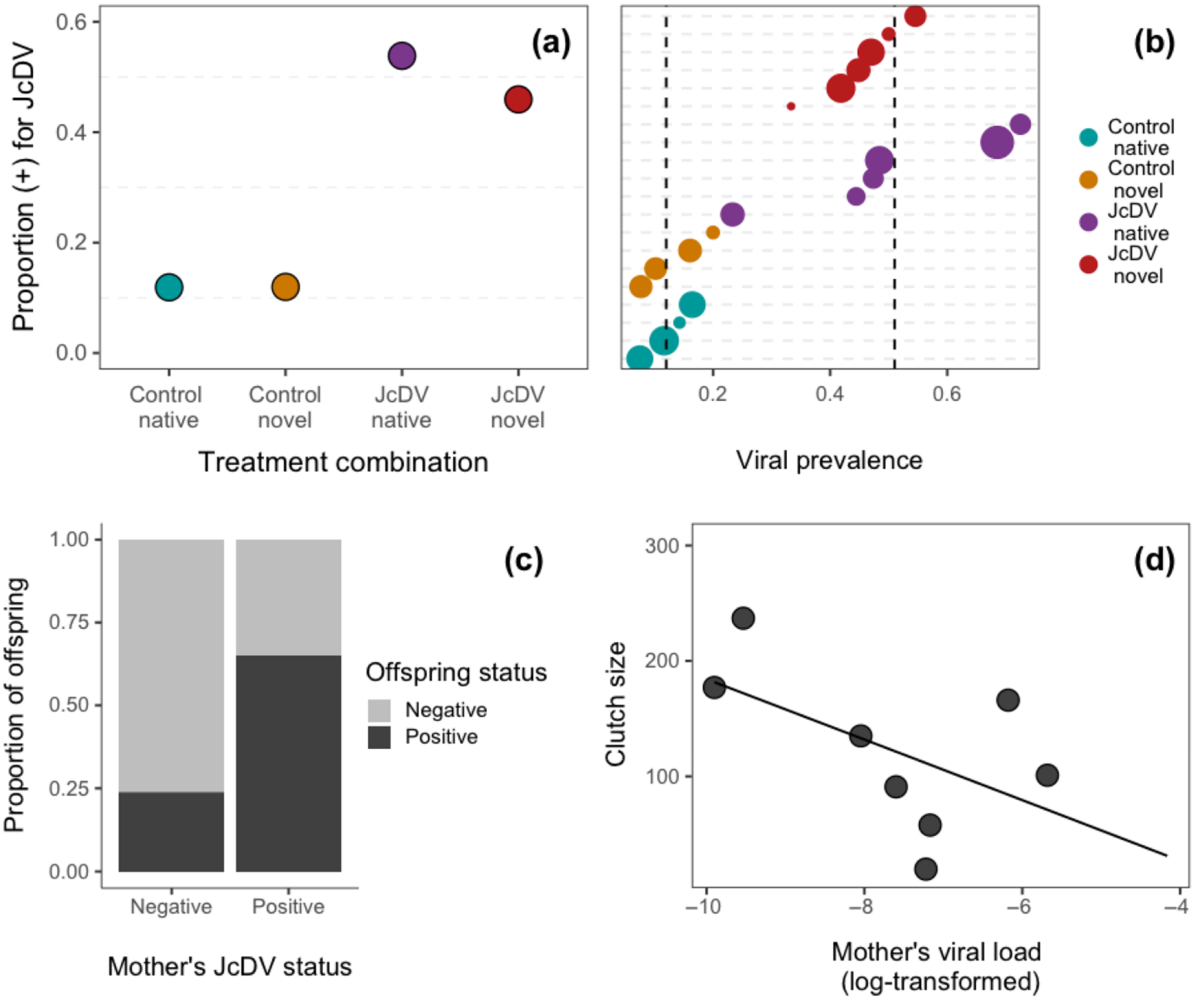

FIGURE 2 (a) Mean final viral prevalence in each of four treatment-level combinations. (b) Final viral prevalence in each natal cluster, where the dots represent the proportion of individuals that tested positive for Junonia coenia densovirus (JcDV). The size of the dot indicates the size of the cluster (number of individuals), and clusters are vertically separated along the *y*-axis. Vertical dotted lines represent mean viral prevalence in the control (left), and inoculated (right) groups. (c) The proportion of offspring that tested positive for JcDV from either JcDV-negative or JcDV-positive mothers. (d) Clutch size (fecundity) predicted by a mother's viral load.

Viral inoculation and host plant did not substantially impact viral loads in individuals that tested positive for JcDV. Membership in the group that received the JcDV treatment slightly increased viral loads, but the 95% credible interval for this effect crossed zero ($\beta = 0.32$, 95% CI = [−1.35, 2.07]), and the host plant had no effect on mean viral loads, ($\beta = 0.00$, 95% CI = [−1.63, 1.68]). There was an effect of life stage on viral load, with the highest relative viral loads occurring in the fourth instar, and lowest in the adults ($\beta = -0.45$, 95% CI = [−0.57, −0.32]). Mothers from the first generation that had higher viral loads had reduced fecundity: an order of magnitude increase in a mother's viral load reduced clutch size by 20%; however the 95% CI for this effect slightly overlapped zero ($\beta = -26.44$, 95% CI = [−64.08, 9.66], Figure 2d).

Overall, 41% of individuals feeding on the novel host plant survived to adulthood versus 8% on the native (odds ratio = 102.5, 95% CI = [10.70, 1808.04]), while 29% of control individuals versus 21% of inoculated individuals survived to adulthood (odds ratio = 0.05, 95% CI = [0.00, 0.44]). Probabilities for surviving to adulthood on *P. lanceolata* were 0.42 in the JcDV group, and 0.40 in the control, and on *C. glabra*, they were 0.00 in the JcDV group, and 0.21 in the control (Appendix S1: Figure S2). An order of magnitude increase in viral load was associated with a 37% decrease in survival to adulthood (odds ratio = 0.59, 95% CI = [0.52, 0.67]; Appendix S1: Figure S4).

The first SEM tested the effects of viral inoculation, host plant, and cluster density on horizontal transmission and survival. We found that survival was higher on the novel host and that there were indirect negative effects of JcDV inoculation through higher horizontal transmission in those groups that received the virus treatment. Larger cluster density caused a small increase in viral transmission and was also directly positively correlated with survival (model $\chi^2 = 2.59$, df = 1, $p = 0.108$; Figure 3). In assessing viral loads and survival in the second SEM, the novel host and larger cluster size were associated with a decrease in viral loads, which in turn was associated with increased survival. Similar to the first model, we found that the novel host directly increased survival. JcDV inoculation did not have a direct effect on viral loads in this model (model $\chi^2 = 1.019$, df = 1, $p = 0.313$; Figure 4). The third SEM was consistent with the hypothesis that higher sequestration of IGs lowered viral loads, but there was no host plant dependence. Larger individuals had lower percent sequestered IGs. This model, which only included data for adults (due to available chemical data), compared with previous models including all life stages, showed that JcDV inoculation was associated with higher viral loads, but it did not influence IG sequestration (model $\chi^2 = 0.045$, df = 1, $p = 0.833$; Figure 5). Additional analysis examining the effects of aucubin and catalpol separately is explored in the supporting information (Appendix S1: Tables S2 and S5; Appendix S2).

DISCUSSION

Given the ubiquity of viruses in natural communities, understanding the factors that contribute to variation in transmission is a primary goal not only for disease ecologists but also for population and community ecologists. Viruses and other pathogens have measurable impacts on insect populations, and quantifying these impacts can contribute to understanding population dynamics. Furthermore, understanding the implications of viral transmission on survival and fecundity can lead to data-driven predictions for how infection will impact population maintenance and growth. However, pathogens are only one selective force that acts on populations of insects; for phytophagous insects, interactions with their host plants are paramount to fitness, and can mediate their responses to pathogens. Therefore, tritrophic interactions involving insects, pathogens, and host plants must be considered to account for biotic factors that influence insect population dynamics. There is a rich body of literature examining host plant-mediation of interactions with insect parasitoids and/or predators (Hansen et al., 2017; Singer et al., 2004; Sznajder & Harvey, 2003).

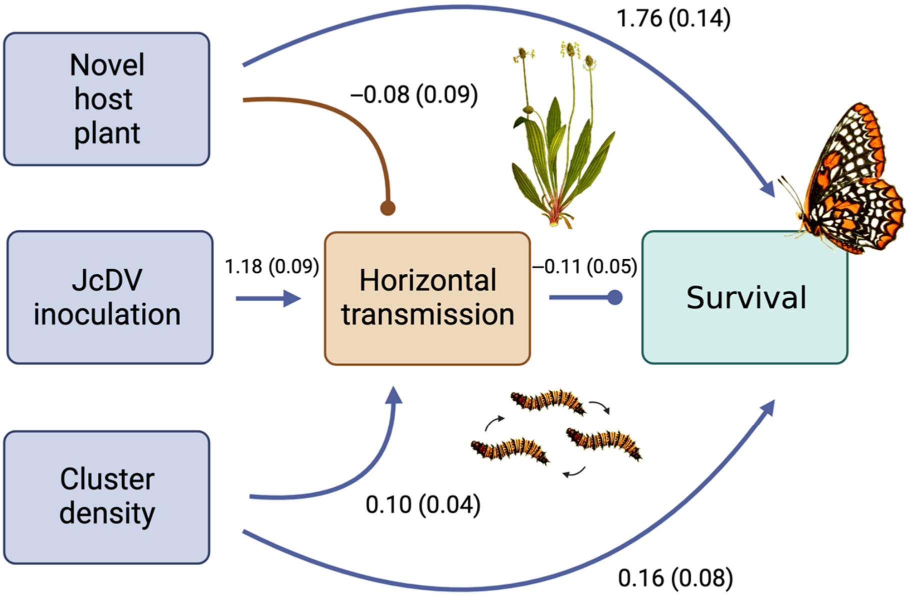

FIGURE 3 The effects of novel host plant use, Junonia coenia densovirus (JcDV) inoculation, and nest cluster density on horizontal transmission (final probability of testing positive for JcDV), and survival throughout the lifespan from the second instar to adulthood. Standardized path coefficients represent the relative strength of the relationship with standard errors given in parentheses. Arrowheads represent positive effects, while dots represent negative effects. Paths that are colored blue represent coefficients with $p \leq 0.05$, while orange paths have a p-value greater than 0.05. Brandon Collins created digital artwork of *Euphydryas phaeton* and *Plantago lanceolata* from original illustrations by William Henry Edwards and Carl Axel Magnus Lindman.

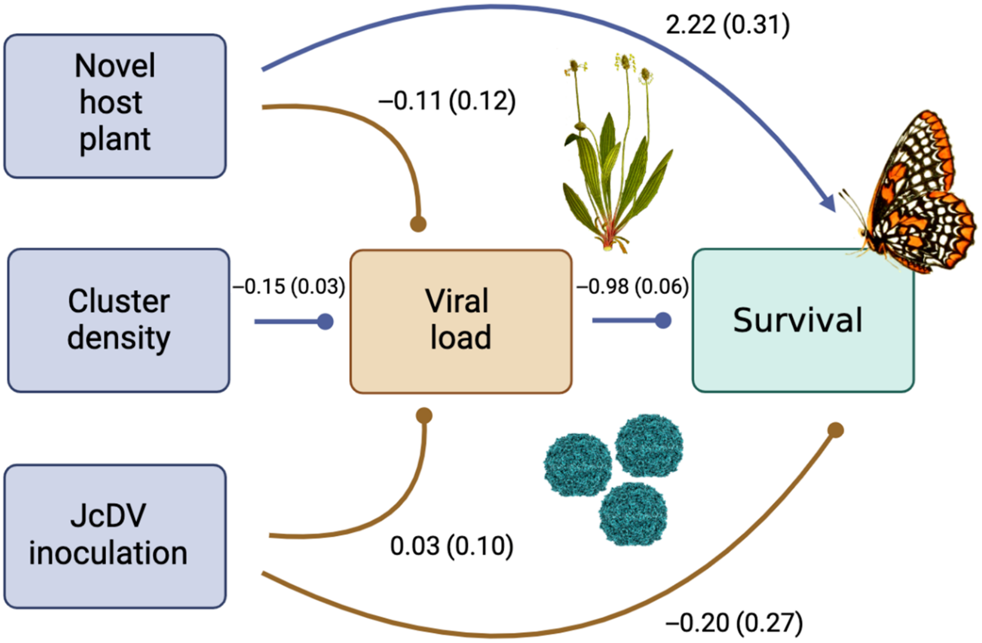

FIGURE 4 The effects of novel host plant use, Junonia coenia densovirus (JcDV) inoculation, and nest cluster density on relative viral loads (the relative amount of JcDV DNA to insect DNA) and survival throughout the lifespan from second instar to adulthood. Standardized path coefficients represent the relative strength of the relationship with standard errors given in parentheses. Arrowheads represent positive effects, while dots represent negative effects. Paths that are colored blue represent coefficients with $p \le 0.05$, while orange paths have a p-value greater than 0.05. Brandon Collins created digital artwork of *Euphydryas phaeton* and *Plantago lanceolata* from original illustrations by William Henry Edwards and Carl Axel Magnus Lindman.

In recent years, this body of work has expanded to include host plant effects on pathogens (Elderd et al., 2013; Mason et al., 2019; Muchoney et al., 2023), and insect immunity (Gallon & Smilanich, 2023; Singer et al., 2014; Smilanich et al., 2009). In our study, we documented a possible advantage to a host range expansion by a native herbivore: protection against pathogens. Specifically, we found that the use of a novel host plant dampened viral transmission and boosted the survival of caterpillars exposed to a virus. This indicates to us that a plant that is typically inferior for caterpillar fitness (Bowers et al., 1992) may in fact be selectively advantageous in a tritrophic context.

Transmission

Understanding different modes of transmission is necessary for determining the demographic impacts of pathogens in wild populations. It is evident that JcDV can spread horizontally, which is of potential importance for gregarious species. Inoculation by JcDV at an initial prevalence of 20% of each cluster resulted in an average of 51% of individuals testing positive for JcDV, whereas the uninoculated groups had a final viral prevalence of 12%. The presence of the virus in the uninoculated groups is most likely the result of vertical transmission of the virus since the parental generation was reared in the laboratory at CU Boulder, and inoculated and uninoculated groups were kept in separate laboratories at UNR with strict protocols to avoid cross-contamination. Furthermore, this experiment provides evidence of vertical transmission in this system. Thus, a background JcDV infection was most likely present in the uninoculated groups, but at a much lower prevalence.

Consuming the novel host plant (*P. lanceolata*) appears to have a small negative effect on horizontal transmission. Moreover, the variation in final viral prevalence was much larger in clusters feeding on the native host plant with two clusters showing high prevalence and one cluster at low prevalence (Figure 2b). This high variation in the native host plant may reflect differences in individual host plant quality including secondary chemistry which can have an impact on viral outcome (see below), although we were unable to explicitly test this hypothesis in our study. A recent study examining JcDV in wild populations of Baltimore checkerspots by Muchoney et al. (2022), did not find host plant differences in viral prevalence, which indicates the role of host plant may be exacerbated in a laboratory setting. We found there was a positive effect of cluster density on the probability of testing positive for JcDV, indicating that horizontal transmission may be greater in denser aggregations of caterpillars, a

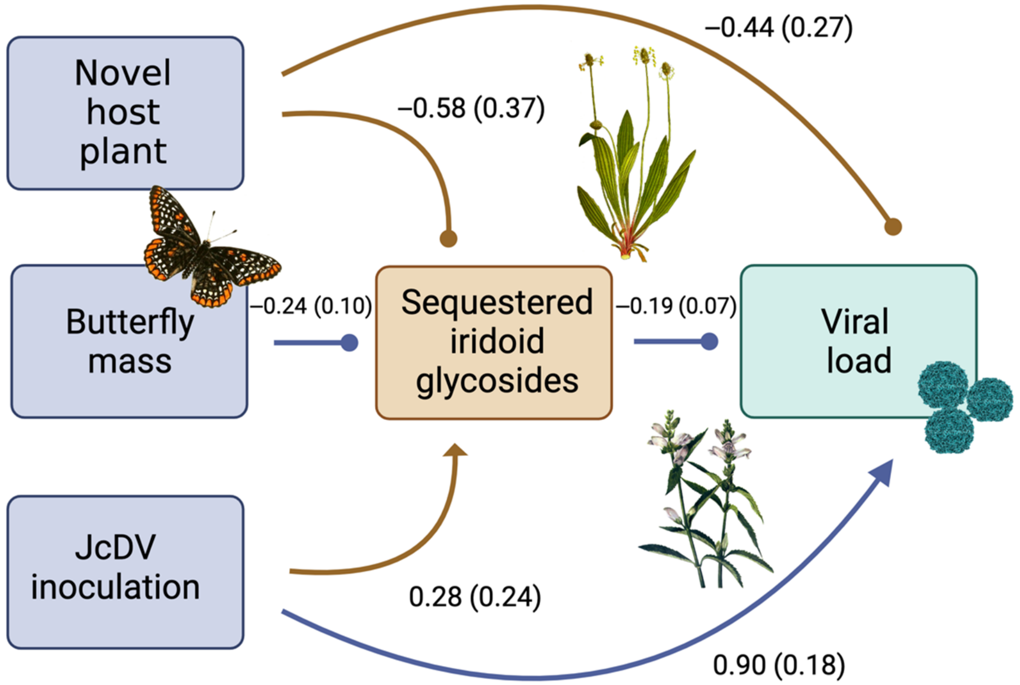

FIGURE 5 The effects of novel host plant use, Junonia coenia densovirus inoculation, and body mass on the concentration of sequestered iridoid glycosides (% dry weight) and viral loads in adult butterflies. Standardized path coefficients represent the relative strength of the relationship with standard errors given in parentheses. Arrowheads represent positive effects, while dots represent negative effects. Paths that are colored blue represent coefficients with $p \leq 0.05$, while orange paths have a p-value greater than 0.05. Brandon Collins created digital artwork of *Euphydryas phaeton*, *Plantago lanceolata*, and *Chelone glabra* from original illustrations by William Henry Edwards, Carl Axel Magnus Lindman, and Mary Vaux Walcott.

phenomenon for which there is mixed evidence in other insect-pathogen systems (D'Amico et al., 1996; Dwyer, 1991; Liebhold et al., 2013; Reeson et al., 2000). Surprisingly, although individuals in denser groups were more likely to contract JcDV, cluster density also had a negative effect on viral loads, indicating they may be more resistant to its effects, suggesting a type of density-dependent prophylaxis where insects reared in larger groups display greater resistance to pathogens through increased immunity (Goulson & Cory, 1995; Reeson et al., 1998; Ruiz-González et al., 2009).

Furthermore, the consistent presence of JcDV in the uninoculated group in this and other studies prompted us to look for evidence of vertical transmission. We found that JcDV-positive status in the mother is a strong and reliable predictor of whether the offspring will test positive for the virus, which supports our hypothesis that JcDV can be transmitted through vertical and horizontal routes of transmission; a phenomenon found in other insect viruses such as nucleopolyhedroviruses that can regulate pest outbreaks (Myers & Cory, 2016; Vilaplana et al., 2010). It has been proposed that utilizing both transmission modes may promote the persistence of a pathogen in the host population (Cory, 2015). It is possible that JcDV is similarly maintained in host populations through both modes of transmission, especially if it is sensitive to UV degradation such as other densoviruses (Miller et al., 1999).

Survival

Our study found complete mortality during and immediately following diapause in caterpillars using the native host plant that had received the JcDV inoculum. Previously, overwintering survival in *E. phaeton* has been shown to both increase (Brown et al., 2017) and decrease (Abarca et al., 2019) on the novel host, indicating that the host plant alone may be insufficient to explain substantial discrepancies in survivorship. Through SEM, we found that both higher viral transmission and higher viral loads were associated with decreased survival, and that higher survival on the novel host plant was, in part, mediated through reduced viral loads. Therefore, increased mortality on the native host likely occurred due to higher viral loads and frequencies of infected individuals, although these do not fully account for the strong host plant effects on survival. Previous work examining JcDV in wild-caught *E. phaeton*, did not find a host plant-dependent effect on survival (Muchoney et al., 2022). However, this study did find that individuals with higher JcDV loads had decreased survival probabilities, as also seen in our study. Additionally, recent work on a confamilial butterfly, *Anartia jatrophae*, found that when experimentally inoculated with JcDV, caterpillars using *P. lanceolata* were nearly six times more likely to survive than those using the primary host plant, *Bacopa monnieri* (Muchoney et al., 2023). This study supports our hypothesis that using novel host plants may be adaptive for insects experiencing selective pressures from pathogens. In addition to mortality through viral infection and host plant effects, JcDV exerted sublethal effects on *E. phaeton* in our study through reduced fecundity; females with higher viral loads had fewer offspring. Reduced fecundity can significantly impact net reproductive rates (Rothman & Myers, 1996), and could represent another mechanism that impacts the population dynamics of wild *E. phaeton*.

Host plant chemistry

It is evident that host plant chemistry plays a primary role in defense against this pathogen. Muchoney et al. (2022) found that increased sequestration of IGs resulted in lower loads of JcDV in *E. phaeton* larvae regardless of the host plant. This prior result was supported in the current study in our adult butterflies, showing that the protective effect of sequestration carries through metamorphosis. These results are similar to those found in a monarch-parasite interaction where sequestration of cardenolides from milkweed host plants reduced parasite burden in the adults (Sternberg et al., 2012).

The mediating effects of host plants on interactions between insects and pathogens are complex (Myers & Cory, 2016) and are seldom considered in the context of insect fitness and population dynamics (Vidal & Murphy, 2018). An inductive inference from this experiment is that when challenged by a pathogen, an herbivore's primary host plant may render it more vulnerable to the pathogen, even if it is superior for other aspects of fitness. Previous studies with *E. phaeton* have found costs related to feeding on the novel host plant, *P. lanceolata*, including diminished growth rates, lower survival, and increased palatability to predators (Bowers, 1980; Bowers et al., 1992). In the context of these interactions, Baltimore checkerspots' oviposition preference on the native host plant aligns with the "preference-performance hypothesis" in which mothers select host plants that ensure optimum offspring performance (Bowers et al., 1992; Gripenberg et al., 2010). Yet, populations of Baltimore checkerspots persist and even thrive on the novel host plant *P. lanceolata* (Brown et al., 2017). Here, we have explored an alternative explanation as to what may contribute to persistence on a novel host plant: enhanced fitness when exposed to a pathogen. The high transmission we observed suggests that this virus could play a significant role in regulating wild populations of *E. phaeton*, and that transmission and mortality may be attenuated on the novel host plant. However, wild conditions are likely to buffer these effects through heterogeneity in the occurrence of the virus in the environment (D'Amico et al., 2005), genetic resistance of insect hosts, through behaviors such as self-medication or avoidance (De Roode et al., 2013; Eakin et al., 2015), and through individual host plant quality. In addition to manipulative experiments, studies of viruses in wild populations of insect herbivores can help to resolve the relative importance of viruses and other pathogens as top-down selective agents, versus host plants as bottom-up selective agents (Singer et al., 2004). Our results suggest that, to fully understand the impacts of pathogens on herbivorous insect populations, we must consider the impacts of their food plants. Ultimately, understanding these complex interactions provides insight into the evolution of diet breadth in insect herbivores by supporting the hypothesis that natural enemies impose selective pressures on herbivores that may expand or restrict their host plant range (Bernays & Graham, 1988).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Christensen, 2023) are available in Zenodo at https://doi.org/10.5281/zenodo.10800561.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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