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A Laterally Transferred Viral Gene Modifies Aphid Wing Plasticity

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SUMMARY

Organisms often respond to changing environments by altering development of particular traits. These plastic traits exhibit genetic variation; i.e., genotypes respond differently to the same environmental cues. Theoretical studies have demonstrated the importance of this variation, which is targeted by natural selection, in adapting plastic responses to maximize fitness [1, 2]. However, little is known about the underlying genetic mechanisms. We identify two laterally transferred genes that contribute to variation in a classic example of phenotypic plasticity: the pea aphid's ability to produce winged offspring in response to crowding. We discovered that aphid genotypes vary extensively for this trait and that aphid genes of viral origin are upregulated in response to crowding solely in highly inducible genotypes. We knocked down expression of these genes to demonstrate their functional role in wing plasticity. Through phylogenetic analysis, we found that these genes likely originated from a virus that infects rosy apple aphids and causes their hosts to produce winged offspring [3]. The function of these genes has therefore been retained following transfer to pea aphids. Our results uncover a novel role for co-opted viral genes, demonstrating that they are used to modulate ecologically relevant, plastic phenotypes. Our findings also address a critical question about the evolution of environmentally sensitive traits: whether the genes that control the expression of plastic traits also underlie variation in plasticity. The genes we identify originated from outside aphids themselves, and thus, our work shows that genes formerly unrelated to plasticity can fine-tune the strength of plastic responses to the environment.

RESULTS AND DISCUSSION

Pea aphids (*Acyrthosiphon pisum*) exhibit a textbook example of phenotypic plasticity, wherein crowded conditions trigger the production of winged rather than wingless offspring. Both morphs are genetically identical to each other and to their mothers due to parthenogenetic reproduction. As in other wing dimorphic insects, winged aphids can disperse to new environments but produce fewer offspring than their wingless counterparts, which leads to a clear trade-off between reproduction and dispersal [4]. The fitness of a particular clone depends on the ability to appropriately sense environmental conditions and produce wingless offspring with high fecundity at low densities or to produce winged offspring when an environment is deteriorating.

We first characterized the variation in wing plasticity present in an aphid population. We used a panel of 192 aphid lines with unique genotypes collected in Ithaca, NY (Chung and Douglas, unpublished data). We raised aphids from each genotype at low density, subjected them to crowding (12 aphids) in a dish for 12 h, and then counted the percentage of winged offspring they produced over the 24 h after crowding. Aphid genotypes exhibited the full range of phenotypic variation, from near 0% to 100% of winged offspring produced in response to the crowding treatment (Figure 1A). This distribution indicates polygenic control of this variation. We then focused on a panel of 10 "highly inducible" and 10 "weakly inducible" genotypes, which we confirmed produce high or low levels of winged offspring in response to crowded but not solitary conditions (Figure 1B).

We investigated whether a higher degree of plasticity comes with a fecundity cost. Highly and weakly inducible genotypes did not differ in their overall fecundity ($\chi^2 = 0.95$, 1DF, $p = 0.33$; Figure 1C) but did differ in how crowded conditions influenced fecundity (treatment × highly versus weakly inducible phenotype; $\chi^2 = 27$, 1DF, $p = 2.3 \times 10^{-7}$). These results suggest that the plastic response itself is costly to aphids, since crowding led to a reduction in the number of aphids born to highly inducible genotypes, but it did not have a similar effect on weakly inducible genotypes. These fecundity costs are in addition to the transgenerational costs of producing low-fecundity winged offspring [5], stressing the importance to an aphid clone of appropriately responding to environmental cues.

To explore the mechanistic basis of variation in plasticity, we sequenced transcriptomes from highly and weakly inducible aphid genotypes (aphids from 10 genotypes pooled per phenotype) under both solitary and crowded conditions (Table S1), and we identified genes differentially expressed in response to crowding. Four genes were differentially expressed in both highly and weakly inducible genotypes (Figures 1D and 1E; Table S2), indicating that some aspects of the response to crowding are shared among genotypes. More importantly, an additional nine

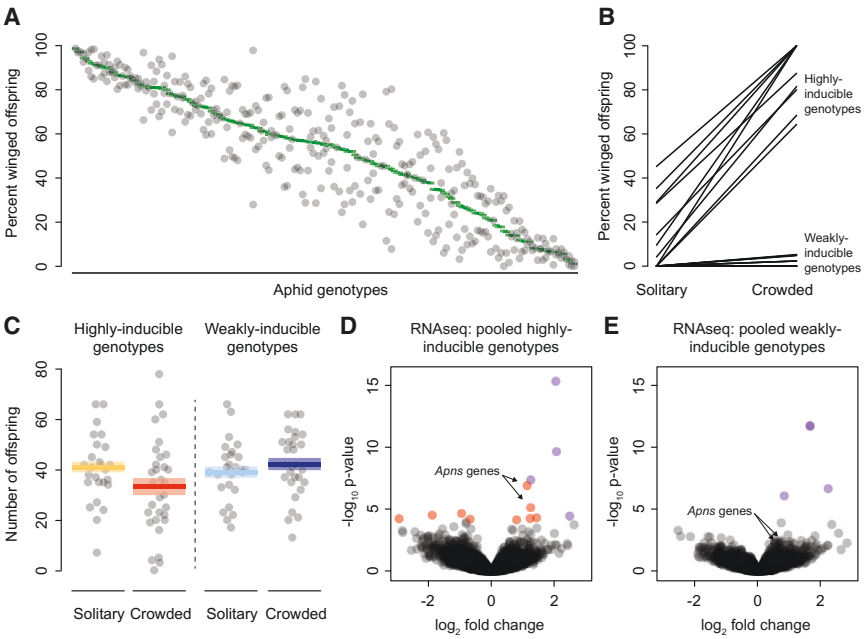

Figure 1. Genetic Variation in Aphid Plasticity

(A) The percentage of winged offspring produced by crowded aphids of 192 genotypes. For each genotype (x axis), a green bar shows the mean of two experimental replicates shown by the gray points.

(B) A panel of 10 highly and 10 weakly inducible genotypes was exposed to solitary or crowded conditions, shown on the x axis. The y axis shows the percentage of winged offspring, with each genotype represented by a black line.

(C) The fecundity of each of the 10 highly and 10 weakly inducible genotypes in response to solitary and crowded conditions is shown. The number of offspring born to each group of three aphids on a single plant produced in the 48 h after treatment is shown along the y axis. The gray points are experimental replicates. The mean of each combination of phenotype and treatment is shown with a colored bar, with standard error represented by the lighter colored boxes.

(D and E) Volcano plot resulting from RNA sequencing of pooled highly inducible genotypes (D) or weakly inducible genotypes (E). Within each plot, the x axis shows the $\log_2$ fold change of each expressed gene in the aphid genome, with higher expression in crowded conditions to the right, and the y axis shows the negative $\log_{10}$ of the p value. Four genes that were statistically significantly differentially expressed by both pools of genotypes (FDR < 0.1) are shown in purple (note that two points are largely overlapping in the weakly inducible plot). An additional 9 genes, which were significantly differentially expressed only in highly inducible genotypes, are shown in red.

genes were differentially expressed only in highly inducible genotypes (Table S2), revealing that some effects of the genetic variation for this plasticity can be discerned at the transcription level. Our highly inducible differentially expressed gene list was remarkably similar to a previous RNA-seq study of a highly-inducible pea aphid genotype, with five genes overlapping despite different experimental conditions [6] (Table S2).

We focused specifically on two of the genes exclusively upregulated by highly inducible genotypes, which we call *Apns*-1 (Figure 2A; ACYPI085607) and *Apns*-2 (Figure 2B; ACYPI36509). The putative proteins of both genes contain a "parvovirus nonstructural protein NS1 superfamily" conserved domain (domain E-value; *Apns*-1: 2.09e-07, *Apns*-2: 4.42e-14). The presence of this viral domain suggests that these genes could be the result of lateral gene transfers into the aphid genome. Both genes are within pea aphid genomic scaffolds and therefore appear to be true genome integrations. To confirm this finding, we used reads from Nanopore sequencing of a pea aphid genotype different than that used in the aphid genome project. Both *Apns* genes show contiguity with aphid sequence, with single long reads spanning both the *Apns* and nearby aphid genes (Figure S1).

To measure expression of these genes in individual genotypes (rather than in pooled samples as in the RNA-seq above), we repeated the crowding assay and used qRT-PCR to measure gene expression in the 10 highly and 10 weakly inducible genotypes. We found that highly inducible genotypes upregulate both *Apns* genes more strongly in response to crowding than do weakly inducible genotypes (Figures 2C and S2A; LMM on ΔC_T values; *Apns*-1: $\chi^2 = 15.6$, 1Df, $p < 0.001$; *Apns*-2: Figures 2D and S2B, $\chi^2 = 5.47$, 1Df, $p = 0.019$), confirming our RNA-seq data. We also found that the expression of both genes is enriched in heads relative to whole-body samples (Figure 2E; Wilcoxon rank-sum tests on ΔC_T values; *Apns*-1: W = 16, p = 0.029; *Apns*-2: W = 16, p = 0.029).

To determine the function of these genes in the aphid plastic wing response, we used RNA interference (RNAi, Figure 2F) to knock down expression. We used three lines (not used above) that were known from previous studies to be highly inducible (using similar protocols, lines SSC3, BK10, and LSR1-01 produced 92.3%, 82.2%, and 62.0% winged offspring in response to crowding, respectively [7]). We injected dsRNA of *Apns*-1 into uncrowded aphids, exposed aphids to crowded conditions, and measured the percentage of winged offspring born to aphids 48-72 h after injection. Because of the similarity of the two *Apns* genes, dsRNA generated from *Apns*-1 cDNA sequence led to the knock down of both viral genes, resulting in a 42% and 43% reduction in expression of *Apns*-1 and *Apns*-2, respectively (Figure 2F; Wilcoxon tests on ΔC_T values; *Apns*-1: W = 86, p = 0.029; *Apns*-2: W = 111, p = 0.0045). dsRNA injection significantly reduced the proportion of winged offspring born to aphids from two of the three genotypes tested (Figure 2G; Wilcoxon tests; SSC3: W = 15, p = 0.15; BK10: W = 27, p = 0.018; LSR1-01: W = 44, p = 0.040), demonstrating that this laterally transferred putative viral gene has a functional role in aphid wing plasticity. We repeated the experiment using the same genotypes but instead crowded aphids before dsRNA injection (Figures S2C–S2H). This method produced qualitative similar results, but the effects were not statistically significant.

Having established a role for the *Apns* genes in pea aphid wing plasticity, we next explored the origin of these putative viral genes in the pea aphid genome. Densoviruses are single-stranded DNA viruses with small (4–6 kb) genomes that are related to parvoviruses and infect a wide diversity of arthropods [8]. Two examples of densoviruses infecting aphids have been

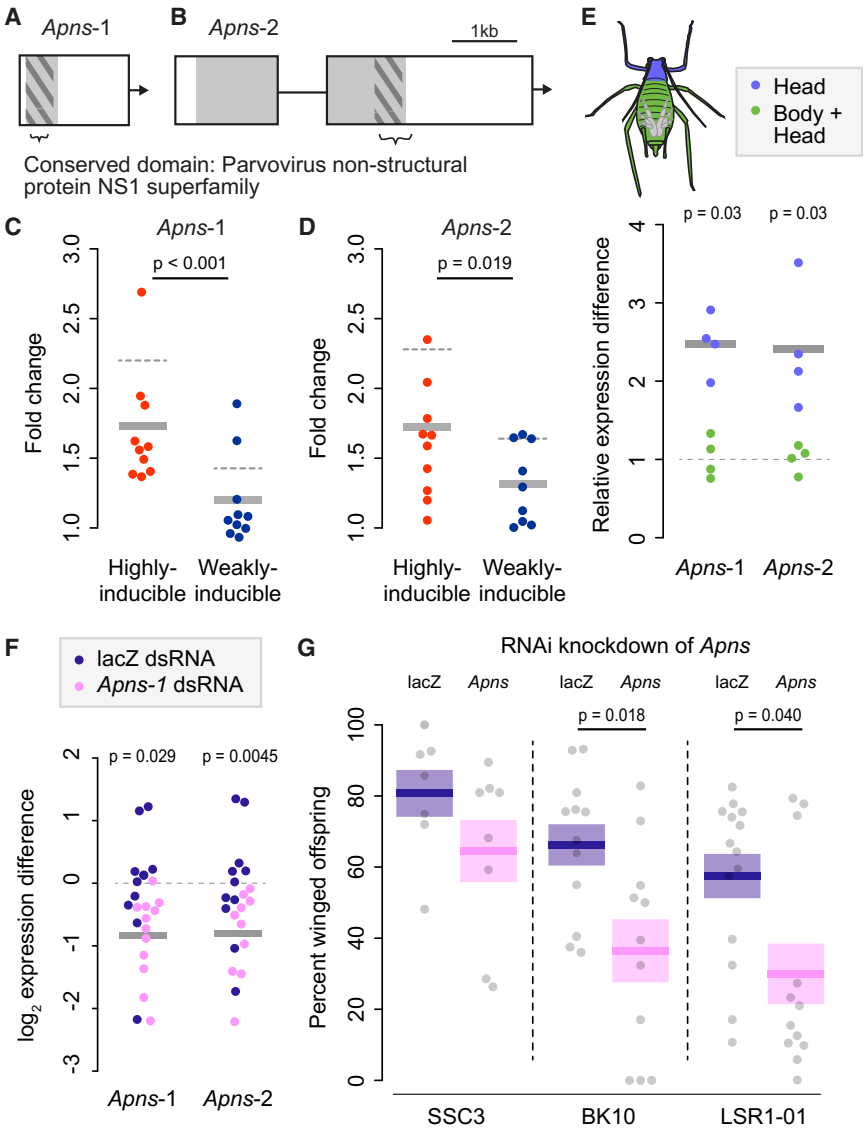

Figure 2. Differential Expression and Function of *A. pisum* Non-Structural Proteins

(A and B) Models of *Apns*-1 (ACYPI085607) and *Apns*-2 (ACYPI36509). The conserved domain is bracketed at the bottom of each figure, and the open reading frame is shown in gray. The region used for phylogenetic alignment is shown by dark gray hashes. A scale bar for both genes (1 kb) is shown at the top.

(C and D) The fold change in gene expression of *Apns*-1 (C) and *Apns*-2 (D) as measured by qRT-PCR in response to crowding relative to solitary conditions, with each genotype shown by a single point (averaged across biological replicates). The mean difference across the 10 genotypes for each phenotype is shown by a solid gray bar, with the differential expression value from the RNA-seq shown with dotted gray line.

(E) Expression levels of the *Apns* genes in heads versus whole-body samples.

(F) The results of RNA interference (RNAi) knockdown of the *Apns* genes as measured by qRT-PCR. The y axis shows the $\log_2$ expression difference of each sample relative to the average expression of control (lacZ-injected) aphids. The gray bars show the average expression difference of the *Apns*-1 dsRNA-injected aphids.

(G) The results of expression knockdown on the percentage of winged aphids. We conducted three replicates of the experiment, each with a different aphid genotype, shown along the x axis. The percentage of winged offspring born to groups of four aphids is shown on the y axis. Treatment (injection with dsRNA from lacZ [control] or *Apns*-1, expected to affect both *Apns* genes) is shown along the top of the figure. Each gray point represents one biological replicate, and the boxes show the mean ± standard error of each combination of experiment × treatment.

See Figures S1 and S2 for more information.

investigated: one from the peach potato aphid, *Myzus persicae* [9], and one from the rosy apple aphid, *Dysaphis plantaginea* [3]. We performed a phylogenetic reconstruction of arthropod densoviruses and found that the pea aphid genes *Apns*-1 and *Apns*-2 clustered within the densovirus sequences and cluster most closely with *D. plantaginea* densovirus (DplDNV) (Figure 3A; alignment, Figure S3A). This phylogenetic placement is consistent with the genes originating via lateral gene transfer.

Densovirus genes, including both structural and nonstructural proteins, have previously been found to be integrated and expressed in the genomes of pea aphids [12] and several other aphid species [13]. We therefore performed a further phylogenetic analysis using publicly available sequences of annotated aphid genes with homology to densoviruses (Table S3) [14, 15]. This analysis again showed that the pea aphid genes grouped with most similarity to DplDNV (Figure 3B, compare to the species tree in Figure 3C), again suggesting that these genes resulted from a lateral gene transfer from a DplDNV-like densovirus. This virus is efficiently transmitted from rosy apple aphids (*D. plantaginea*) to their offspring (vertical transmission) and is described as a viral mutualist. This is because the production of winged offspring in rosy apple aphids is dependent on infection with DplDNV, and viral infection increases host mobility and promotes dispersal [3]. Densovirus-free rosy apple aphids do not produce winged offspring even in response to crowding and poor host plant conditions. Intriguingly, therefore, the free-living densovirus most closely related to the integrated densovirus genes in the pea aphid causes the production of winged aphids. We note, however, that support for the branch containing the *Apns* genes and DplDNV was low in both analyses, and an alternative possibility is that the *Apns* genes originated from a lateral transfer from *Myzus persicae* densovirus or an uncharacterized aphid virus. We performed an extension of this analysis using aphid sequences from two additional genomes (*Diuraphis noxia* and *M. cerasi*) that again supported our finding that the *Apns* genes originate from LGT of an aphid densovirus and might further suggest that sequences closely related to DplDNV have repeatedly integrated into multiple aphid genomes (Figure S3,

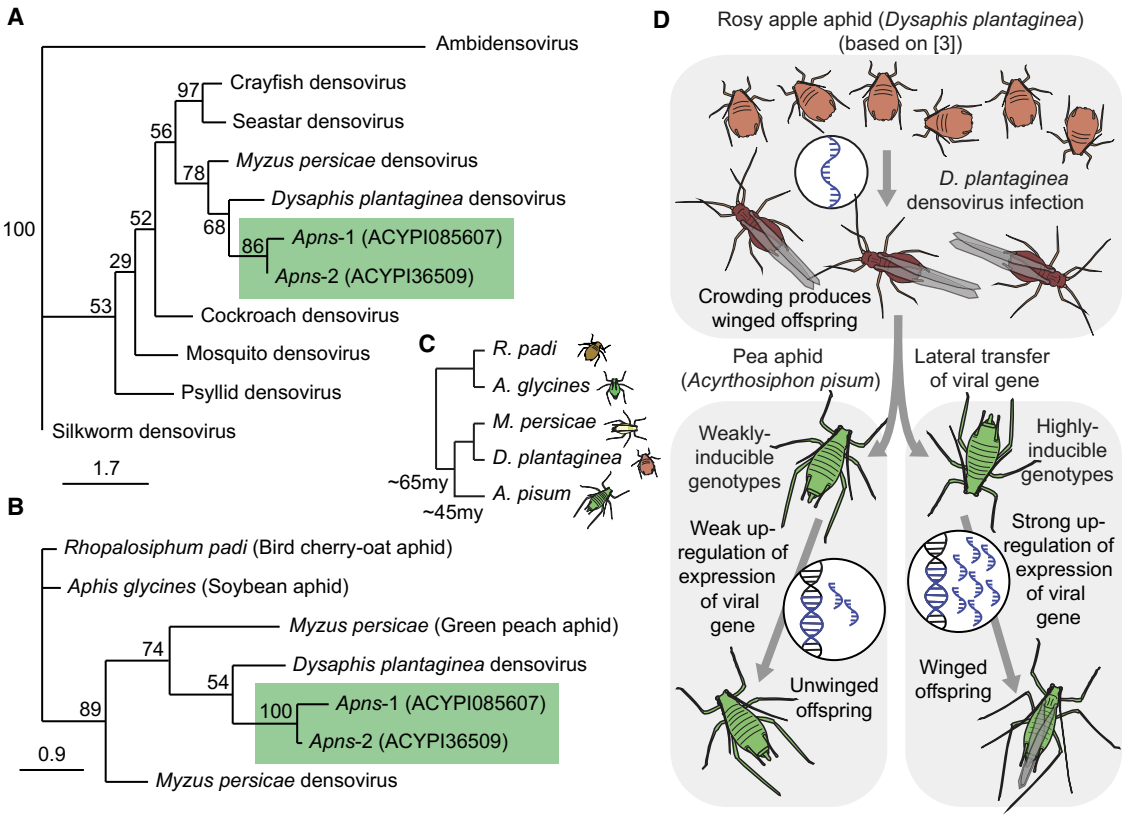

Figure 3. Origin of Pea Aphid Densovirus Genes and Their Retention of Function

(A) Unrooted, bootstrap consensus tree showing the protein phylogenetic relationships among densovirus sequences from different invertebrate hosts, related viruses (parvovirus and ambidensovirus), and pea aphid *Apns*-1 and *Apns*-2.

(B) Unrooted, bootstrap consensus tree created from amino acid directed nucleotide alignments showing the phylogenetic relationships among densovirus sequences (from *Myzus persicae* and *Dysaphis plantaginea*) and aphid homologs (*Rhopalosiphum padi*, *Aphis glycines*, *Myzus persicae*, and pea aphid *Apns*-1 and *Apns*-2). For both trees, only nucleotide sequences alignable with the shorter pea aphid copy (*Apns*-1) were used. 1,000 bootstrap trees were generated with maximum likelihood.

(C) Species tree for the aphid species referred to in Figure 3B. The relationships among species are inferred from trees in Kim et al. [10] and Hardy et al. [11]. Divergence times are based on [10].

(D) Model illustrating densovirus gene domestication and subsequent retention of function. See Figure S3 and Table S3 for more information.

though see below). Future study of the timing and origin of the *Apns* genes in aphids is needed.

We suggest that the densovirus genes have the same effect on winged morph induction in the pea aphid that densovirus infection has in *D. plantaginea*, and these genes likely have retained their function after introduction into the pea aphid genome (summarized in Figure 3D). It is unclear whether *D. plantaginea* densovirus actively induces winged forms in its host using this nonstructural protein or whether *D. plantaginea* responds to viral infection by producing winged offspring, and future work is needed to uncover the precise mechanism by which these proteins act. However, densovirus nonstructural proteins are generally involved in virus replication and transcriptional activation of capsid genes [16]. Non-structural proteins of vertebrate parvoviruses can activate transcription factors and induce epigenetic modifications in hosts through histone acetylation [17]. *Apns*-1 and *Apns*-2 may, therefore, induce transcription of genes related to wing morph determination, potentially acting in the brain given the enriched head gene expression levels.

Lateral gene transfers are an important source of phenotypic change in prokaryotes, but only recently have we begun to appreciate the frequency and importance of lateral transfers from microbes in eukaryotic evolution [18]. Most laterally transferred DNA is not expressed by eukaryotic hosts, and it is quickly inactivated or eroded. Examples of functional lateral gene transfer are therefore uncommon, but some prominent examples come from the integration of viruses into host genomes [19] and from the transfer of DNA from vertically transmitted bacteria to their animal hosts [20]. Our study provides a clear example of a functional lateral gene transfer from a vertically transmitted viral partner to its host.

Genetic variation in plastic traits has been documented in a diversity of taxa, from natural variation among isolates of the nematode *Pristionchus pacificus* in the production of dimorphic adult mouth forms [21] to predator-mediated plasticity in the shape of *Chthamalus anisopoma* barnacles [22]. This variation has been theorized to be important in adapting plastic responses to fitness optima [2]. Our results provide insight into the molecular mechanisms underlying genetic variation in phenotypically plastic traits.

This lateral gene transfer event appears to be part of a modulation or "fine tuning" of the sensitivity of the plastic response to the environment. The densovirus gene insertion event is much more recent than the evolution of the wing plasticity itself, which is ancient to aphids and common to many major aphid groups [23]. This finding therefore sheds light on an important question about how phenotypically plastic traits evolve: whether genes that underlie variation in a plastic trait also control expression of the trait or whether genes from outside of these developmental pathways are co-opted to modify the strength of a plastic response to environmental cues. The answer in this case is clearly the latter. Not only are the *Apns* genes from outside the developmental genetic pathway for the aphid wing plasticity, they also are from outside aphids themselves.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.05.041.

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AUTHORS CONTRIBUTIONS

B.J.P. and J.A.B. designed the research; B.J.P. performed and analyzed the plasticity experiments, transcriptome library prep and analysis, qRT-PCR, and RNAi; J.A.B. performed the phylogenetic analysis; J.A.B. funded the work; and B.J.P. and J.A.B. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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