Functional genomics in the wild: A case study with paper wasps shows challenges and prospects for RNA-interference in ecological systems

Journal:	<i>Genome</i>
Manuscript ID	gen-2017-0066.R1
Manuscript Type:	Article
Date Submitted by the Author:	18-Aug-2017
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Is the invited manuscript for consideration in a Special Issue? :	Ecological Genomics
Keyword:	RNA-interference, ecological genomics, Polistes, eusociality, castes

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Title:

Functional genomics in the wild: A case study with paper wasps shows challenges and prospects for RNA-interference in ecological systems

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Abstract

RNA-interference (RNAi) is a useful tool to assess gene function by knocking down expression of a target gene, and has been used successfully in domestic and laboratory organisms. However, RNAi for functional genomics has not fully extended into ecological model organisms in natural environments. Assessment of gene function in the wild is important, because gene function can be environmentally and context-dependent. Here, we present a case study using RNAi to assess gene function in wild paper wasps *Polistes metricus*, to test roles for two candidate genes (*NADH dehydrogenase* and *Retinoid and fatty acid binding protein*) in the development of reproductive castes. Previous studies showed these genes are upregulated in larvae that become queens compared to workers, but this pattern was reversed in the laboratory, making field-based studies necessary. We orally administered dsRNA to larvae in field colonies and found evidence of a short-term knockdown followed by a compensatory rebound in expression for *RfaBp*. We also observed the predicted worker-like decrease in lipid stores in *NADHdh*-dsRNA treated wasps, suggesting a possible role for *NADHdh* in caste development. We discuss our results in the context of challenges for using RNAi for functional genomics in ecological model organisms in the field.

Keywords: RNA-interference, ecological genomics, *Polistes*, eusociality, castes

Introduction

RNA-interference (RNAi) is a powerful tool for functional genomics, which utilizes organisms' innate molecular machinery to knock down expression of target genes (Agrawal et al. 2003). RNAi has been used successfully in a wide variety of domesticated or laboratory plants and animals (Price and Gatehouse 2008), but is also of great potential value in ecological genomics because of its applicability to ecological model organisms. In theory, all that is required to conduct an RNAi experiment is some knowledge of the expression pattern and sequence of a target gene, forgoing the need for whole genome sequences, controlled breeding, gene transformation technology, and large resources/research networks. In addition, because RNAi can be administered directly into organisms at specific developmental stages and target tissues, it has the potential to provide specific and potentially more realistic information about gene function than full germ line knockouts.

Given these advantages, there has been increasing interest in using RNAi for ecological model systems to assess gene function in organisms' natural environments. This type of approach is important, as genes' effects may be highly context-dependent and inferred gene functions may turn out to be ecologically irrelevant if done in a highly artificial laboratory setting. Also, phenotypic traits of interest and their associated gene expression patterns may change under laboratory conditions if traits are plastic (Jandt et al. 2015), thus it is important to pair more highly controlled studies of gene function in the laboratory with field-based approaches.

69

70 Despite the compelling reasons for using RNA-interference in natural settings, studies utilizing
71 RNAi in the field have been relatively few. Most of these have focused on practical applications
72 of RNAi, rather than using RNAi to assess gene function. For example, there have been field
73 trials of dsRNA to combat viral infections in honey bees (Maori et al. 2009, Hunter et al. 2010)
74 and genetically engineered plants producing dsRNA to control pest insects (Baum et al. 2007,
75 Price and Gatehouse 2008). There are numerous examples of successful utilization of dsRNA to
76 assess gene function related to social caste development in honey bees and termites (Amdam et
77 al. 2003, Zhou et al. 2008, Patel et al. 2007, Mutti et al. 2011, Kucharski et al. 2008, Li-Byarlay
78 et al. 2013, Kamakura et al. 2011), but many of these have focused on lab-reared individuals. In
79 honey bees, there have been successful uses of RNAi to study individual genes' effects on
80 behavior and social organization in the field (Nelson et al. 2007, Ament et al. 2012, Antonio et
81 al. 2008). However, honey bees are a semi-domesticated species and genetic background,
82 colony structure, and age of individuals can be controlled much more readily than in truly wild
83 populations.

84

85 In general, RNAi to assess gene function in ecological model organisms is challenging, as there
86 is a large amount of variation in the efficacy of RNAi across taxa, different tissue types, and
87 different target genes (Scott et al. 2013, Wynant et al. 2014). Arguably, studies of gene function
88 in the field using wild populations are the most challenging, as field environments are highly
89 variable with many uncontrolled factors. Also, studying natural populations means potentially
90 high levels of genetic variation, which can lead to more variable results due to variable genetic

4

backgrounds (Scott et al. 2013). To date, there is a glaring lack of studies assessing gene function in an ecologically relevant context for wild populations of ecological model species, and need for more open discussion in the published literature about the challenges and inherent issues with transferring RNAi technology from lab to field.

Here, we describe research utilizing RNAi to test the role of a candidate gene in the regulation of social caste development in natural populations of an ecological model organism, *Polistes* paper wasps. Like other eusocial insects, *Polistes* paper wasp females have a caste system with reproductive queens and non-reproductive workers. However, castes are flexible in *Polistes* and influenced by environmental and social factors during both larval development and adulthood (Hunt 2007). The genetic and developmental basis of caste development has been of great interest, because the presence of castes is the defining feature in the major transition in evolution from solitary to colonial life. Thus, there have been numerous studies aiming to identify the genetic basis of caste differences in a variety of eusocial species, from termites to bees to ants to wasps (reviewed in Toth and Rehan 2017). In honey bees, RNAi studies in the laboratory have identified genes with causal associations related to caste differences, implicating differential gene expression related to DNA methylation (Kucharski et al. 2008, Li-Byarlay et al. 2013) and insulin signaling (Patel et al. 2007, Mutti et al. 2007).

Polistes wasps are an especially interesting system to study the genetic basis of caste differences because they are primitively eusocial, meaning they possess smaller colonies and castes are reversible and less defined than species with more derived sociality (Hunt 2007). There are now

genomic resources for several *Polistes* species (reviewed in Jandt and Toth 2015) and RNAi methods in the field have already been established, though without success in affecting phenotype (Hunt et al. 2011). Previously, studies from three different *Polistes* species have consistently shown strong caste-related differences in gene expression during larval development. Although *Polistes* castes are ultimately determined during adulthood via dominance interactions, and queens and workers are morphologically similar, seasonal and feeding differences set up a strong caste-bias during the larval stage (Hunt 2007). Along with this differential development, several genes related to mitochondrial metabolism and oxidation-reduction processes, with upregulation of these genes in larvae collected late in the colony cycle, which will typically emerge as pre-overwintering queens (called “gynes”) (Toth et al. 2010—*Polistes metricus*, Standage et al. 2016—*Polistes dominula*, Ferreira et al. 2013—*Polistes canadensis*).

In *Polistes metricus*, one particular oxidation-reduction gene, *NADH dehydrogenase*, emerged as the single most consistent “candidate” gene associated with gyne development across four studies (Toth et al. 2007, Toth et al. 2010, Berens et al. 2015, Jandt et al. 2015). Another gene, *RfaBp* (retinoid and fatty acid binding protein), was also strongly associated with the gyne caste in multiple studies of *P. metricus* in both larvae and adults (Toth et al. 2007, Toth et al. 2010, Hunt et al. 2010). However, previous studies showed that expression of caste-related genes, including the two candidates described above, in the closely related *Polistes fuscatus* changed to show reversed caste-related expression patterns under laboratory rearing (Jandt et al. 2015), necessitating studying gene function in a natural, field-based setting. We thus aimed to

determine whether an RNAi based knockdown of *NADH dehydrogenase* or *RfaBp* in the field would lead to the development of more worker-like phenotypic traits. Because NADH dehydrogenases are involved in mitochondrial energy production, and RfaBp is a chaperone protein for fatty acids, we predicted that one or both of these genes could affect nutritional aspects of caste development, in particular lipid deposition.

The goals of this study were threefold. First, we aimed to assess the potential causal roles of *NADH dehydrogenase* and *RfaBp* in caste development in *Polistes metricus*. We hypothesized that knockdown of either gene in gyne-destined larvae would lead to the development of lower lipid stores, a defining worker-like phenotypic trait (Toth et al. 2009). Second, we assessed the efficacy of RNAi in the field at two timescales, examining expression of target genes immediately following several days of treatment, and expression several days post-treatment. We did this because it has been widely reported that knockdowns may be transient in nature, thus we wanted to address the importance of timing on RNAi efficacy. Third, we provide an assessment of challenges and pitfalls of RNAi in the field revealed by this experiment, with suggestions about how to overcome these issues in the future.

Materials and methods

Synthesis of dsRNA

Primers used in the dsRNA synthesis protocol were designed for *RfaBp* and *NADH*

dehydrogenase (NADHdh) based on contigs from the *P. metricus* EST database and RNA-sequencing data (Toth et al. 2007, Berens et al. 2015). Commercially available primers for *GFP* were also used as a control (all custom primer sequences provided in the Supplementary Material Table 1). The primers were fused with the T7 sequence and PCR was performed on *P. metricus* DNA using these primers. The PCR product was purified using the QIAquick™ PCR Purification Kit (Qiagen). From this product, we prepared dsRNA using the RiboMAX™ T7 RNA Production System (Promega) and resuspended it in water (Hunt *et al* 2011).

Animal collection and treatment

In spring of 2011, wooden nest boxes were posted on metal poles at Chichaqua Bottoms in Maxwell, IA, and *P. metricus* foundresses were allowed to found nests naturally inside the boxes, resulting in 8 available nests. All nest boxes were of similar construction and were placed in a single, relatively homogenous grassy field. In each nest, approximately 5-10 individual larvae were treated, but not all survived to pupation, and some were removed from subsequent analysis upon determining that they were males *via* anatomical inspection after collection (Cotoneschi et al. 2007). In all experiments, the location and contents (egg, larva (including instar), pupa, or empty) of each cell in the comb of all nests was noted on a hexagonal grid, providing detailed nest maps before the start of treatment. We updated the maps daily and recorded the dates of initial treatment, stage of each larval instar determined by head capsule width (Rembold et al. 1980), date of pupation, and any individuals that disappeared from the nest (presumed dead).

In order to target gyne-destined larvae, the time period of the experiment (July 21, 2011 -

August 4, 2011) occurred well after the emergence of the first workers on each colony. During the summer, the naturally founded nests were monitored daily and all 2nd and 3rd instar larvae were fed 1 uL of a solution of 30% honey in water with 0.5ug/mL dsRNA. Feeding stopped when they reached 4th instar. This time period (2nd and 3rd instar out of 5 total larval instars) was chosen because it appears to be a sensitive period for caste determination in many wasps, likely including *Polistes* (Jeanne 2009). This time period was also better for logistical reasons; 1st instar cannot be hand fed due to their extremely small size, and 4th and 5th instar larvae regurgitate a large amount of saliva (which is imbibed by adults), making it challenging to ensure consistent treatment of older larvae. Each larval instar lasts between 2-3 days (Hunt 2007), thus, wasps were orally treated on a daily basis with dsRNA for a range of 4-6 days. All larvae in a given nest were treated with the same dsRNA (*GFP*, *RfaBp* or *NADHdh*) to prevent transfer of dsRNA between treatments by trophallaxis with adults.

After the treated individuals pupated between 4-6 days later, they were collected for analysis and immediately frozen on dry ice. In 2011, final sample sizes were 8, 7, and 5 (from 3, 2, and 3 nests) for the *GFP*, *NADHdh*, and *RfaBP* dsRNA treatments, respectively. We expected caste related phenotypic differences to be most easily detected in this stage because all lipids needed for adult development are present at this point.

The experiment was repeated in 2013, but with slightly altered methods because we found no target gene expression knockdown when examining pupae collected in 2011. The experiment in 2013 aimed to examine gene expression immediately after treatment. Thus, 13 naturally established *P. metricus* nests from the same site were used. For the 2013 experiment, we used the same duration of dsRNA treatment as in 2011, but instead of allowing larvae to

reach pupation, larvae were collected onto dry ice when they reached 4th instar, one day after dsRNA treatment ceased. In 2013, nests were monitored and were fed daily (for 4-6 days) with the three different dsRNA treatments as in 2011. The start date of the treatment was July 10, 2013, and the end date was July 16, 2013. In 2013, final sample sizes were 9, 4, and 5 individuals (from 5, 4, and 4 nests) for the *GFP*, *NADHdh*, and *RfaBP* dsRNA treatments, respectively.

Lipid Extraction

Lipids from the same focal pupae described above from the 2011 experiment larvae (after removal of the heads) were quantified using a colorimetric assay with a sulphophosphovanillin reagent as in Toth et al. (2009). Briefly, headless wasp pupae were homogenized in 2:1 chloroform: methanol, the sample was left to extract overnight, then filtered and adjusted to a constant volume. Then, a sulphophosphovanillin reagent was added to a subsample of each extract, and the resulting color change was quantified on a multiwell spectrophotometer. Each set of samples was run with a standard curve of known quantities of pure cholesterol, allowing for an estimation of lipid quantity in each sample.

Real Time qRT-PCR

Samples were removed from deep-freeze at -80C and maintained on dry ice for processing. Pupal heads were removed from each sample using a razor blade and kept frozen until RNA extraction. RNA from heads was used because previous studies had established caste differential gene expression in heads (Berens et al. 2015, Jandt et al. 2015). Also, heads were

chosen because *Polistes* caste associated genes often show differential expression in brains (Toth et al. 2009) and because using whole animals or fat bodies would have prevented lipid quantification from the same animals. RNA was extracted using the Qiagen RNEasy kit, following manufacturer's instructions. RNA was quantified and quality checked using a NanoDrop, then reverse transcribed using Superscript II (Invitrogen). We then used real-time quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) with Sybr Green (Applied Biosystems) for gene expression estimation using a Roche Light Cycler. We used gene specific primers to amplify each of our two target genes, *RfaBp* and *NADHdh*, as in previous studies on *Polistes metricus* (Toth et al. 2007, Hunt et al. 2010). For each sample, we also analyzed the expression of two internal control genes, using the external control *mCherry* and internal control *Rps8* for 2011 data, and two internal controls (*Elongation factor-1 α* (*Ef-1 α*) and *actin*) for 2013 data. qRT-PCR data were normalized to internal control genes, as described in previous studies of *Polistes metricus* (Jandt et al. 2015, Toth et al. 2007).

Data Analysis

We used GraphPad software to analyze and graph data from mortality, lipid, and qPCR data. Mortality was compared using Fisher's Exact tests, and lipid stores were compared using an ANOVA, with Tukey post hoc tests. Real time PCR data were run in triplicate, and mean values for each sample were used in the analysis. qPCR data for each of the genes of interest were normalized to one (for 2011 data) or two (for 2013 data) internal control genes that have been previously used in *Polistes* and other social insects, *Rps8*, *actin* and *Ef-1 α* (Jandt et al. 2015, Toth et al. 2007). The treatments were compared to the *GFP* control to find $\Delta\Delta\text{CT}$ and the fold

difference in expression. Due to non-normality of the qPCR data, we used non-parametric Kruskal-Wallis ANOVA tests for differential gene expression.

Results

Effects of dsRNA treatment on mortality and body mass

In order to rule out that our treatments resulted in severe disruption of organismal function, we compared mortality rates (based on number of larvae “missing” from nests). Over the course of the entire experiment in 2011, we found no evidence of elevated mortality in either of target gene dsRNA treated larvae compared to controls fed *GFP* control dsRNA (Figure 1, *NADHdh*: Fisher’s Exact test $p = 0.2143$, *RfaBp*: Fisher’s Exact test $p = 0.4550$). High levels of larval mortality in the reported range are typical for *Polistes* wasps in our study populations (Jandt et al. 2015). We also found no evidence of an effect on total body mass (ANOVA, $F = 1.16$, $p = 0.34$).

Effects of dsRNA treatment on lipid stores

We assessed lipid stores of pupae from the 2011 experiment, which were fed target gene dsRNA throughout the 2nd and 3rd instars of their larval development and subsequently collected several days later as pupae. We found significant differences in total lipid stores between the dsRNA

treatment groups (ANOVA, $F = 11.62$, $p = 0.0006$) and lipid as a percentage of total body mass (ANOVA, $F = 7.487$, $p = 0.0047$), and post-hoc tests revealed significantly lower lipid stores in *NADHdh* dsRNA treated compared to *GFP* dsRNA control treated wasps ($p < 0.01$), but no difference between the *GFP* dsRNA control and the *RfaBP* dsRNA treated wasps (Figure 2).

Effects of dsRNA treatment on gene expression levels

We examined the effects of dsRNA feeding on gene expression levels of target genes over two different time scales. In 2011, we collected individuals after pupation, after they had been fed dsRNA during second and third larval instars, then larvae had a rest period of 4-6 days, until they were eventually collected as pupae with newly spun cocoons. These were the same larvae that we conducted lipid analyses on (Figure 2). The results indicated no significant knockdown for either of the genes of interest on this longer time scale (*NADHdh*: ANOVA $p = 0.17$, *RfaBp*: ANOVA $p = 0.64$, raw data in Supplementary Material Table 2).

To examine whether knockdown may have occurred on a shorter time scale than we measured in 2011, we performed an additional experiment in 2013 to examine gene expression immediately after treatment (larvae were collected as 4th instar larvae, one day after receiving multiple days of treatment during their 2nd and 3rd larval instars). In this case, we did observe significant knockdown of *RfaBp* expression when wasps were fed dsRNA for *RfaBp* compared to dsRNA *GFP* controls (Kruskal-Wallis ANOVA $F = 6.032$, $p = 0.049$, Figure 3A), with no significant

knockdown of the non-target gene *NADHdh*. For our second target gene *NADHdh*, there was a weak trend toward reductions in both genes of interest (*NADHdh* and *RfaBp*) compared to *GFP* controls, but these differences were not statistically significant (Kruskal-Wallis ANOVA $F=5.358$, $p = 0.069$, Figure 3B).

Discussion

To our knowledge, this study is one of the first attempts to use RNA-interference to examine gene function in a wild population in a field setting (Hunt et al. 2011). We had strong reasons for examining gene function in the field, as our previous work indicated gene expression for members our focal genus, *Polistes* paper wasps, is highly sensitive to environmental conditions, and lab gene expression patterns are greatly perturbed in comparison to gene expression under field conditions (Jandt et al. 2015). We tested two possible candidate genes for involvement in producing caste-related traits in *Polistes metricus* during larval development, and found some suggestive evidence that one gene, *NADHdh*, may be involved in lipid deposition, one of the main diagnostic traits of the gyne caste in *Polistes* (Toth et al. 2009). Although our study shows promise for functional genomics in the wild, there are many challenges and caveats that must be addressed.

The results for *NADHdh* in this study were highly suggestive, but not conclusive. We did find a

phenotypic effect of *NADHdh*-dsRNA treatment-- as predicted, these wasps had lower, more worker-like lipid stores compared to controls. However, we failed to find a knockdown of gene expression for *NADHdh* when expression levels in these wasps were assayed several days after the dsRNA treatment had ended. On a shorter time frame, we found a trend toward lowered gene expression in the *NADHdh* treated wasps compared to controls one day after dsRNA treatment, but this effect was only marginally significant. Thus, our results are not conclusive regarding the role of *NADHdh* in the development of caste-related traits in *Polistes*. Our results (from both *NADHdh* and *RfaBp*) hint at strong time dependency of the gene expression knockdown, and we suggest the lack of a significant knockdown could be related to the time we sampled. It is possible that we missed the time in which the strongest knockdown occurred, perhaps even within hours of administering the treatment. Indeed, previous studies of RNA-interference in insects have demonstrated the highly transient effect of expression knockdown, which may even be limited to within a few hours after the administration of the treatment (Scott et al. 2013, Wynant et al. 2014).

We did produce a significant knockdown of our second target gene, *RfaBp*, at least at the shorter time scale immediately following dsRNA oral administration. However, we failed to find any effects on lipid stores in the *RfaBp* treated wasps. This result was similar to a previous study in *Polistes metricus*, which also achieved a significant knockdown of *hexamerin 2*, with no obvious phenotypic effects (Hunt et al. 2011). While it is possible that these genes are not causally related to caste differences in wasps, it is also feasible that the lack of a significant phenotypic effect could possibly relate to the social nature of feeding in *Polistes*; if the treatment led to

nutritional stress, adults could have compensated by increasing provisioning to stressed larvae. Alternatively, *RfaBp* and *hexamerin 2* could affect other aspects of caste-related phenotypes in wasps which were not examined. For example, *RfaBp* was previously shown to be differentially expressed in *Polistes metricus* adult females performing different foraging and reproductive behaviors (Toth et al. 2007), so perhaps this gene could have some functions in caste-related brain development and/or behavior. Future studies should examine the effects of this gene (and others) on a wider variety of caste related traits, including adult behavior and reproductive physiology.

Although our results show some promise for using RNA-interference to assess gene function in wild populations, this study also highlighted several challenges. For example, not surprisingly, we saw high variation in gene expression levels in the wasps we sampled, thus making it difficult to detect significant differences in gene expression even when trends were apparent. This high variation in gene expression could be related to genetic variation among our sampled wasps (Toth et al. 2014, Henshaw 2013) suggests moderately high genetic variation in *Polistes metricus* populations. There could also be environmentally-induced variation in gene expression due to wasps' experiencing different social, nutritional, and even abiotic environments across different nests. Another challenge of working in the field with wild populations are limited sample sizes, as it may be impossible to find large numbers of age-, sex-, and life-cycle-matched individuals in wild populations to reach the levels of replication needed to demonstrate a target gene knockdown, in the face of already dynamic patterns of gene expression. Another challenge, not unique to working in the field, is the highly time-specific effects of the knockdown. Our results

are in line with work in other study systems that show that the timing of the RNA-interference knockdown may be quite transient, thus making verification of a knockdown challenging, even when there is a clear phenotypic effect of the treatment as in our study. This problem can be compounded by working with wild populations in which the number of individuals accessible for study is highly limited in a given year, making testing of multiple time points difficult.

Despite these challenges, our data suggest that studies of functional genomics in the wild are within reach, and we believe they are definitely worthwhile because of the importance of assessing gene function in an ecological context. In Table 1, we outline several of the challenges of RNA-interference in the wild. Based on our experience from this study, we suggest some possible solutions for dealing with these issues. In addition, we raise additional considerations not directly addressed but implicit in this study (e.g. tracking treated individuals in the field, and best methods of delivery for RNA-interference studies in the field), as well as make suggestions about the types of ecological systems that would be best suited to these types of studies. We acknowledge that each study system is unique, and that not all will face the same challenges nor be able to utilize the same solutions. Nonetheless, if ecological genomics is to continue to push forward into the functional realm, it will be important to increasingly integrate studies of gene function with natural history information, including assessments of causal relationships between genes and phenotypes in field-based, wild populations. In addition, it will be important to pair controlled, laboratory-based studies of gene function (allowing for more power to detect subtle phenotypic effects) with field-realistic tests of gene function (allowing researchers to assess how much single gene effects matter in a natural setting).

Looking ahead into the future, there are other highly promising technologies for functional genomics on the horizon, for example, germ line transformation using transposable elements (e.g. Piggy-bacs in honey bees, Shulte et al. 2014) and use of TALENs (transcription activator-like effector nucleases, Joung and Sander 2013) and CRISPR-Cas9 (clustered, regularly interspaced short palindromic repeats) for gene editing (Ran et al. 2013). Some of these technologies are already being applied to non-model organisms (Bonasio 2015, Kistler et al. 2015, Sugano et al. 2014, Markert et al. 2016), and may be more effective than RNAi in species that have proved recalcitrant to RNAi, including many vertebrates. Although the generation of stable lines of genetically transformed animals may not be feasible or safe in the context of wild populations, there may still be potential for using transgenic mosaics (limiting transmission of germ line mutations to progeny) to assess gene function, or use of genetically modified organisms in contained, semi-natural settings (Markert et al. 2016). In conclusion, there are many challenges still to overcome, but also exciting prospects on the horizon for the future of functional genomics in ecological model systems.

Acknowledgements: This work was supported by a National Science Foundation, Integrative Organismal Systems, Developmental Systems Panel grant to ALT. Jonathan Jackson assisted with field work, and members of the Toth laboratory provided helpful feedback on the project and the manuscript.

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Table 1. Some challenges and possible solutions to using RNA-interference to assess gene function in wild populations.

Challenge	Possible solutions	Best organisms to target
high levels of genetic variation in focal individuals	increased sample size; use single population when possible	abundant species; species that are highly clustered in specific habitats
time dependency of RNAi knockdown	pilot experiments to determine optimal timing; subsample individuals at different times after treatment	species in which individuals can be easily marked and tracked over time in the wild
limited sample sizes	plan for multi-year replication	abundant species; species that are highly clustered in specific habitats
finding treated individuals after release into wild populations	individual marking; use of containment within natural environment	stationary species or species that return regularly to a central place and are easily marked
efficient delivery of dsRNA to wild species under field conditions	oral delivery or delivery in food may be more realistic than injections	species that will readily take food provisions; may be more efficient in early developmental stages (in insects)
highly variable gene expression patterns across individuals	target a gene with consistent, non-tissue specific expression patterns	species with as much prior gene expression data available as possible to assess consistency of expression, tissue specificity

Figure Legends

Figure 1. There were no differences between dsRNA treatment groups in the proportion of individuals that were dead or alive for *P. metricus* larvae treated with dsRNA between either of the target genes and the control *GFP* (*NADHdh*: Fisher's Exact test $p = 0.2143$, *RfaBp*: Fisher's Exact test $p = 0.4550$)

Figure 2. Lipid levels in 5th instar *Polistes metricus* larvae treated with either 1 μ g dsRNA per day of either *RfaBp*, *NADHdh*, or *GFP* (ANOVA, $F = 7.48$, $p = 0.0047$, letters above bars indicate significant differences based on Tukey post-hoc tests, $n=5$ (*RfaBp*), $n=7$ (*NADHdh*), $n=8$ (*GFP*).

Figure 3. Effects of dsRNA treatment on gene expression immediately after oral treatment. (A) Gene expression as measured by qRT-PCR of *RfaBp* (Kruskal-Wallis ANOVA $F = 6.032$, $p = 0.049$) and (B) *NADHdh* (Kruskal-Wallis ANOVA $F = 5.358$, $p = 0.069$) of wasps treated with dsRNA for *RfaBp*, *NADHdh* or *GFP*. Significance difference of post-hoc Tukey pairwise comparisons ($p < 0.05$) is indicated by *. $n=5$ (*RfaBp*), $n=4$ (*NADHdh*), $n=9$ (*GFP*).

Supplementary Information

Supplementary Table 1. Primer sequences used for PCR reactions. All primer sequences were derived from contigs from the *Polistes metricus* EST database (Toth et al. 2007), with the exception of *actin*, which was from the *Polistes dominula* genome (Standage et al. 2016). Previous studies demonstrated the use of this *Polistes dominula* sequence produced robust and reliable PCR results (Jandt et al. 2015). Also note that in previous publications the gene *NADHdh* was referred to as an *oxidoreductase* (Toth et al. 2007), but a more recent search showed the best BLAST hit, more specifically, to be *NADHdh*.

Gene name (forward or reverse primer)	Gene type	Primer sequence
<i>RfaBp</i> - forward	Target	GTCCTCCATCAGTGACAGTTCCT
<i>RfaBp</i> - reverse	Target	TACTACACGTACAGGCCTACTGTTCA
<i>NADHdh</i> - forward	Target	TTAGGAAAGATTATGGCCTTCTATGG
<i>NADHdh</i> - reverse	Target	TGCACAGCCAACAGGAAATG
<i>Actin</i> - forward	Internal control	GTCGTACCACCGGTATTGTATTG
<i>Actin</i> - reverse	Internal control	GCCAAATCGAGACGAAGGATAG
<i>Ef-1α</i> -forward	Internal control	CTCCAGTACCAGCAGCAACAAT
<i>Ef-1α</i> -reverse	Internal control	GCACCTGGACACAGAGATTTC
<i>Rps8</i> - forward	Internal control	CCAGGCGCAGTGCTCTGTA
<i>Rps8</i> - reverse	Internal control	AAACTTGGACCTCAGCGTATTCA
<i>mCherry</i> -forward	External control	GGCTTCAAGTGGAGCG
<i>mCherry</i> -reverse	External control	GGAGGGAAGTTGGTGC

Supplementary Table 2. Raw data from 2011 Real time PCR data for dsRNA treated wasp pupae under three different dsRNA treatments, either one of the target genes (*RfaBp* and *NADHdh*), or the *GFP* control. Data shown refer to mean cycle threshold values (Cp) across triplicate technical replicates for each sample, and quantity estimates are calculated from mean Cp values, based on a standard curve of *Polistes metricus* genomic DNA.

Treatment	MeanCp	Quantity
<i>RfaBp</i>	28.16337641	78.17293637
<i>RfaBp</i>	29.02399017	54.73891387
<i>RfaBp</i>	29.85041186	37.59867783
<i>RfaBp</i>	29.2207727	50.43861411
<i>RfaBp</i>	28.17837175	77.48740362
<i>RfaBp</i>	29.50836347	44.03712183
<i>NADHdh</i>	30.13692167	32.63713284
<i>NADHdh</i>	29.56973207	42.75930943
<i>NADHdh</i>	30.86303518	22.22404725
<i>NADHdh</i>	29.29365537	48.86285722
<i>NADHdh</i>	30.41732681	28.31421722
<i>NADHdh</i>	30.6086383	26.1362737
<i>NADHdh</i>	27.83598602	87.8738625
<i>GFP</i>	29.52338847	44.07996421
<i>GFP</i>	28.23008448	75.97469333
<i>GFP</i>	29.89685719	38.21741354
<i>GFP</i>	28.21346293	76.72474153
<i>GFP</i>	29.2015392	50.92264941
<i>GFP</i>	29.055344	54.16251495
<i>GFP</i>	29.26863119	49.71526899
<i>GFP</i>	29.64701508	41.69921919
<i>GFP</i>	29.43585468	45.67036941

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