Host quality induces phenotypic plasticity in a wing polyphenic insect

Xinda Lin[a,1,2], Yili Xu[a,1], Jianru Jiang[a], Mark Lavine[b], and Laura Corley Lavine[b]

[a]College of Life Sciences, China Jiliang University, 310018 Hangzhou, China; and [b]Department of Entomology, Washington State University, Pullman, WA 99164-6382

Edited by Sean B. Carroll, HHMI and University of Wisconsin, Madison, WI, and approved June 4, 2018 (received for review December 11, 2017)

Food quality is a critical environmental condition that impacts an animal's growth and development. Many insects facing this challenge have evolved a phenotypically plastic, adaptive response. For example, many species of insect exhibit facultative wing growth, which reflects a physiological and evolutionary trade-off between dispersal and reproduction, triggered by environmental conditions. What the environmental cues are and how they are transduced to produce these alternative forms, and their associated ecological shift from dispersal to reproduction, remains an important unsolved problem in evolutionary ecology. In this study, we investigated the role that host quality has on the induction of wing development in a wing polyphenic insect exhibiting strong tradeoffs in investment between dispersal and reproduction, the brown planthopper, a serious rice pest in Asia. As rice plants grow, the short-winged brown planthopper dominates the population, but a shift occurs as the plants mature and senesce in the field such that long-winged brown planthoppers emerge and migrate. It remains unknown how changes in the rice plant induce development of the long-winged morph, despite recent discoveries on the role of the insulin and JNK signaling pathways in wing development. We found that by mimicking the glucose concentration of senescing rice plants, we significantly increased the proportion of long-winged female planthoppers. The effects of glucose on wing morph are additive with previously described effects of density. Our results show that host quality both directly regulates phenotypic plasticity and interacts with other factors such as density to produce the appropriate phenotype for specific environmental conditions.

host quality | phenotypic plasticity | brown planthopper | wing dimorphism | insulin signaling pathway

An organism must be able to rapidly respond to changing environmental conditions or otherwise face significant consequences in survival, reproduction, and fitness (1). Polyphenism, the developmental capacity to couple coordinated expression of alternative suites of morphological, physiological, and behavioral traits with circumstance, is an effective solution to this problem, as it permits an organism to facultatively invest in the production of costly traits only when conditions are appropriate. This rapid response to condition is commonly seen in insects such as aphids and crickets, which face a pronounced allocation tradeoff between flight and reproduction (2–9). During development, these insects either develop into fully winged forms capable of flight or flightless forms that instead allocate resources to reproduction (1, 3, 8, 10–12). Although it has long been evident that wing polyphenic insects couple wing growth with exposure to specific environmental cues, such as increased crowding and/or changing nutrition, the environmental cues that trigger the physiological and genetic mechanisms responsible for alternate patterns of wing growth are less well understood.

Several ecological factors are known to be key inducers of phenotypic plasticity in insects. A critical factor is variation in host quality, which is a well-known cue for insect phenotypic plasticity in growth and form (13–20). The wing polyphenic brown planthopper is a unique system in which to study phenotypic plasticity because of the simple dynamics of the host plant (rice) with the insect. During the early development and growth of the rice plant, host quality is high. Populations of brown planthopper sampled at this time exhibit a high ratio of short-wing to long-wing morphs (21, 22). The short-winged morph is considered to be the reproductive form of the brown planthopper (8, 20, 21–24). However, as the rice plant begins to mature, the soluble sugar content increases to its highest level and the plant begins to senesce (25). This very large increase in glucose has been observed to coincide with an increase in the ratio of the long-winged to short-winged brown planthoppers in field populations (20, 21).

In the field, ratios of long-winged to short-winged brown planthoppers are highest when the rice plant is senescing or near harvest (26–28). Dispersal is dependent on the long-winged morph (8, 20, 22–24). Migration allows brown planthoppers to escape unfavorable conditions and colonize new areas (28, 29). Physiological status and growth stage of the rice plant, temperature, nymph density, and hormones regulating metamorphosis have all been reported to affect the wing morph ratio (22, 24, 26, 30–35).

Recent research has revealed the role of several signal transduction pathways in mediating wing dimorphism in the brown planthopper, i.e., the involvement of the insulin signaling and JNK signaling pathways (36–38), as well as an unidentified pathway induced by wounding that operates, like insulin signaling, through its effect on the transcription factor FOXO (39). However, it has not been shown precisely how, or even if, ecological factors such host quality and density activate pathways such as insulin signaling or JNK in determining wing morph.

Although the correlation between the growth stage of rice plants and the wing morph ratio of brown planthoppers has been described (26, 27, 35), the mechanism linking the two remains unknown. Glucose acts as a key signal transduction molecule in

Significance

Variation in food nutrient content and density are key ecological factors linked to the expression of condition-dependent, adaptive phenotypes such as wing polyphenisms. There is very little known about exactly what the ecological cue is that induces the appropriate insect phenotype in wing polyphenic insects. Our study reveals that glucose concentration of the host plant and insect density directly influence the development of brown planthoppers into either the long-winged migratory morph or the short-winged reproductive morph. This study is a step in linking host quality signals and other factors such as density to the induction of adaptive phenotypes in insects.

Author contributions: X.L. designed research; X.L., Y.X., and J.J. performed research; X.L., Y.X., and J.J. analyzed data; and X.L., M.L., and L.C.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]X.L. and Y.X. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: linxinda@cjlu.edu.cn.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1721473115/-/DCSupplemental.

Published online July 2, 2018.

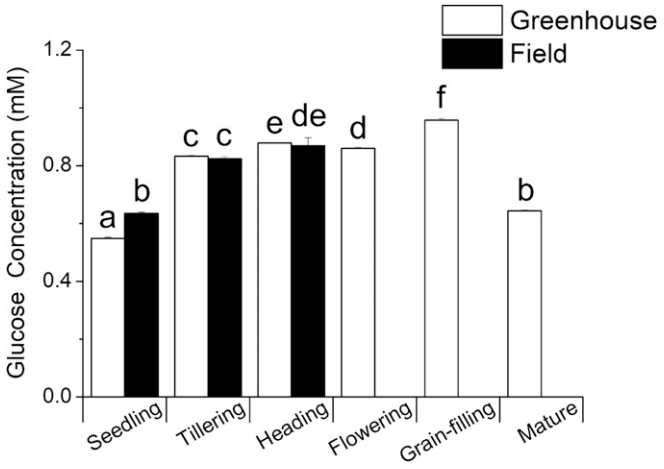

Fig. 1. Variation in glucose levels in rice stalks sampled from the greenhouse and field. Open box, stalk of the rice plant collected from the greenhouse; filled box, stalk of the rice plant collected from the field. Different letters indicate $P < 0.05$. Duncan's multiple-range test was used for comparison. The glucose concentrations of the stem of the rice plant at seedling, tillering, heading, flowering, grain-filling, and milk stages were used. For all samples, $n = 6$.

the regulatory pathways of plants, allowing rapid adaptation to environmental stress and mediating growth and development (30). Glucose is also a molecule critical for animal development, where insulin signaling modulates glucose concentration for cell proliferation, growth, and development. Taken together, these data suggest that glucose concentration in rice plants has a role in regulating wing polyphenism in brown planthoppers.

Here, we tested the effects of host quality on wing polyphenism by artificially altering glucose concentration in rice seedlings, in conjunction with differing densities of brown planthoppers. We further examined interactions between glucose levels and density on wing morph ratio by altering these variables in conjunction with RNAi-mediated knockdown of insulin signaling genes. We found that higher glucose concentration in rice plants resulted in an increase in the proportion of long-winged female (LWF) brown planthoppers. In addition, the effects of glucose concentration on wing morph were found to be additive with density, indicating host quality both directly regulates phenotypic plasticity and interacts with other factors such as density to produce the appropriate phenotype for specific environmental conditions.

Results and Discussion

Rice Plant Glucose Levels Increase with Plant Growth and Development. An important consideration in our artificial elevation of plant glucose concentration is how these levels may correspond to changes in glucose concentration in naturally senescing rice plants. Glucose concentrations in rice plants increase as the plants age and senesce. To compare our manipulated seedlings with naturally occurring changes in rice glucose concentration, we measured glucose concentration during rice plant development in greenhouse and field experiments. We collected rice plants at the seedling, tillering, heading, flowering, grain-filling, and mature stage. The stems were collected, and glucose concentrations were measured. The results showed that, as previously reported (25), the glucose concentration of rice plants increased from the seedling stage through the grain-filling stage (Fig. 1) and decreased at the mature stage as the plant died. The glucose concentrations in field-collected plants were similar to those from the greenhouse (Fig. 1), except at the seedling stage, where the glucose concentration was higher in the field than in the greenhouse plants. Also, we were unable to measure some of the later stages in the field. What is important for our study, however, is that the differences in glucose concentration in early versus late season plants are much greater than what was produced by manipulating rice seedlings in the laboratory. Thus, the effect of the increasing glucose concentrations over time in the field could be predicted to have a much greater effect on wing morph, resulting in the near total shift to the long-winged form typically observed in the field.

Increased Glucose Levels in Host Rice Plants Caused an Increase in the Ratio of LWF Brown Planthoppers. To test the effect of increased glucose content in rice plants on the wing morph ratio of the brown planthopper, we first established that we could produce rice plants with elevated glucose content. We cultured rice seedlings in culture medium supplemented with 1% glucose for 12, 24, and 48 h and compared them to seedlings grown in media without added glucose. We then measured the concentration of glucose in the rice plants at specified time points. The concentration of glucose increased significantly in seedlings supplemented with 1% glucose relative to controls for each time point (Fig. 2). Glucose concentrations were 0.032 μM, 0.033 μM, and 0.061 μM higher than controls after culturing for 12, 24, and 48 h, respectively (Fig. 2).

We then asked whether the increased glucose concentration affected the wing morph ratio of brown planthoppers feeding on those plants. We fed fourth instar nymphs, which is the critical period for morph determination (32), with rice seedlings cultured in either 1% glucose-supplemented or nonsupplemented control media for 48 h and compared the wing morph ratios. Because of the effect of density on determining wing morph in brown planthoppers (22, 33, 34), we also varied the number of nymphs placed in each tube containing a rice seedling, adding 1, 5, 10, or 20 nymphs per tube. Increased density and increased sugar concentration had significant effects on wing morph ratio, but interestingly this was true for female planthoppers only (Fig. 3 *A* and *D*). When planthoppers developed in numbers higher than one per tube (i.e., 5, 10, or 20), there was a significant increase in the number of LWFs relative to short-winged females (SWF). This was true whether the plants were glucose supplemented and

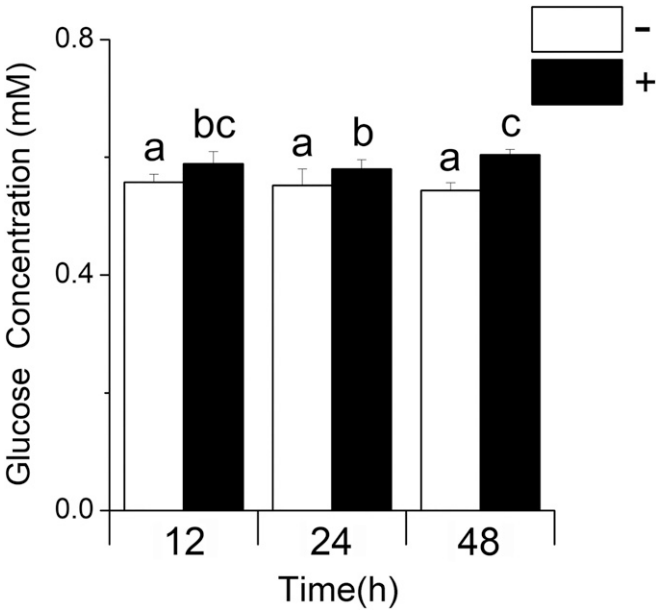

Fig. 2. Glucose supplementation of the rice seedling culture medium results in increased glucose concentration in the rice plant. Glucose concentration was measured after 12, 24, and 48 h of glucose treatment. Different letters indicate $P < 0.05$. Duncan's multiple comparison was used to compare all individual treatments to each other. Significant differences are indicated by different letters. For all samples, $n = 6$.

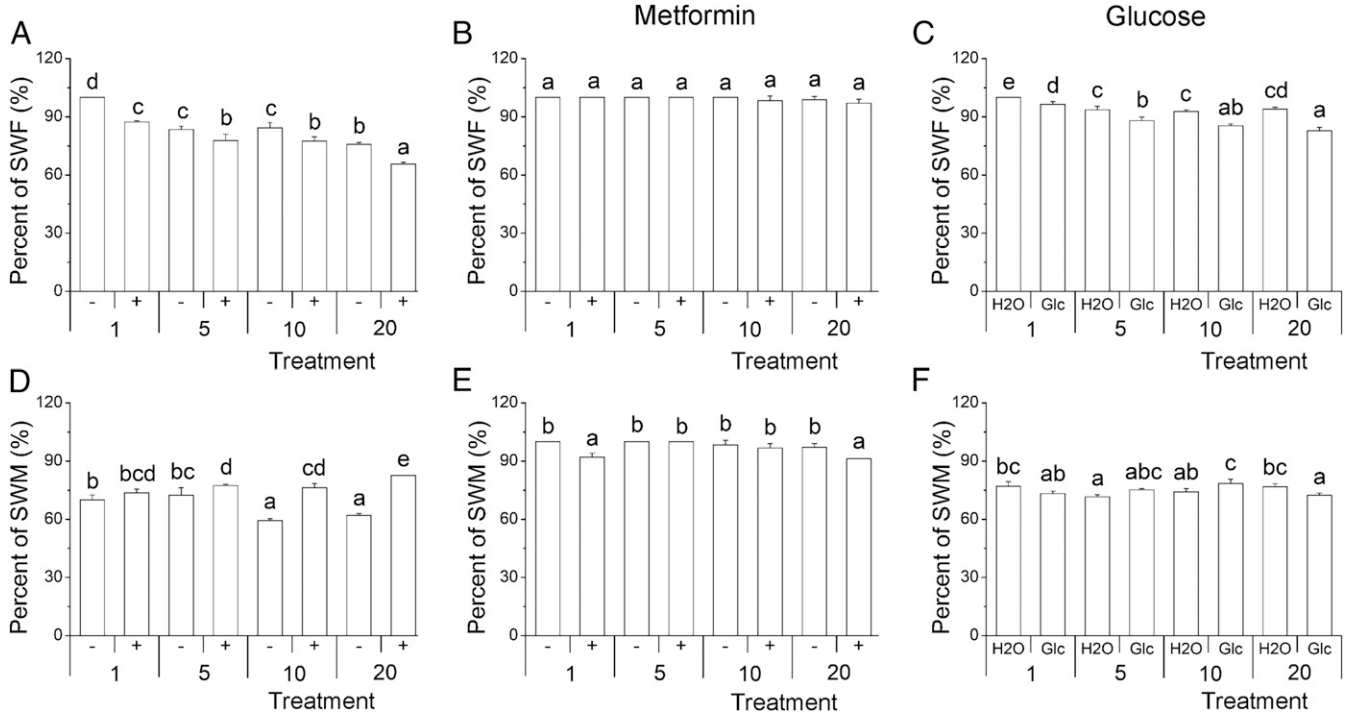

Fig. 3. Glucose supplementation increased the ratio of female long-winged brown planthoppers either indirectly through the rice plant or by direct injection into the insect and was dependent on density. Density was varied from 1, 5, 10, to 20 brown planthoppers per tube. Two-way ANOVAs were performed, and Duncan's multiple-range test was used to differentiate between the means; different letters indicate $P < 0.05$. *A* and *D* are the no treatment control insects that fed on rice seedlings (–) or glucose supplemented rice seedlings (+). The percentage of SWFs decreased significantly (with a concomitant increase in long-wing morphs) in glucose supplemented rice seedlings compared with controls and was significantly affected by density. The percentage of short-winged males (SWMs) was more variable when fed on glucose supplemented rice seedlings but increased significantly with increasing density. The effects of glucose supplementation on wing form in females and males was different and in opposite directions. *B* and *E* are the insects that were injected with metformin. Metformin injection resulted in over 90% short-winged females and males for all densities except for males that were either alone (1) or in groups of 20 supplemented with glucose. *C* and *F* are the insects that were injected with glucose or H_2O. Glucose injected females produced significantly more long-winged morphs and was significantly positively affected by density. Males were not significantly affected by glucose injection or by density. Sample sizes for each experiment are as follows: NC– (n = 105/120/102/156 females, 72/102/172/184 males), NC+ (n = 188/90/93/198 females, 53/53/42/69 males), Metformin– (n = 110/122/100/164 females, 92/114/120/134 males), Metformin+ (n = 92/114/120/134 females, 65/102/126/150 males), H_2O (n = 114/84/136/117 females, 127/117/149/141 males), Glucose (n = 125/78/137/115 females, 153/123/151/142 males). "+" indicates addition of a 1% glucose supplement to the rice seedling culture medium. "–" indicates no glucose was added. N/N/N/N indicates at densities of 1, 5, 10, and 20 per tube.

agrees with previous reports of increased density favoring development into long-winged adults (22, 33, 34). Likewise, an effect on wing morph due to feeding on glucose-supplemented plants was apparent mainly in females (Fig. 3 *A* and *D*). There was a higher proportion of LWF in supplemented versus nonsupplemented plants for all planthopper densities (although the effect was not statistically significant for the 5 per tube density). However, the effects of density and glucose on males was different—there was a significant increase in the long-winged morph only at the highest density, and only in nymphs fed on glucose-supplemented plants. For the males, the only density that differed between glucose-supplemented and controls was 10 leafhoppers per tube—and this differed in the opposite direction as with females, with significantly fewer LWM in the glucose-supplemented group. The results showed that increased density and feeding on plants with higher glucose concentrations significantly increased the proportion of females developing into the long-winged morph but had only minimal effects on males.

Direct Injection of Glucose into Nymphs Increases the Ratio of LWF Brown Planthoppers. We also tested for possible direct effects of glucose on wing morph by injection of glucose directly into the hemocoels of fourth instar nymphs. Direct injection of glucose into the brown planthopper increased the ratio of LWF at all densities (Fig. 3*C*) but had no significant effect on males regardless of density (Fig. 3*F*), consistent with the more natural experiment of the insects feeding on rice plants to obtain dietary glucose.

Inhibition of Glucose Metabolism by Metformin Resulted in Decreased Ratios of Long-Winged Brown Planthoppers. Metformin is a drug commonly used to treat diabetes because of its ability to lower blood glucose levels and lower circulating insulin levels (40–42). We investigated the effect of decreasing glucose production and levels of circulating glucose on wing morph ratio by injecting metformin directly into brown planthoppers during nymphal development (Fig. 3 *B* and *D*). Results of the two-way ANOVA indicated significant interaction effects between glucose and density in the no treatment males ($P < 0.001$, df = 3), and a similar interaction effect was observed after injection of water (both in female and male, $P < 0.01$, df = 3), as well as after injection of metformin (male, $P < 0.01$, df = 3). As predicted, injections of metformin reversed the effect of glucose supplementation on wing morph ratio in females (Fig. 3*B*). In fact, metformin prevented the development of long-wing morphs regardless of density or glucose content of the host plant. Males were slightly less affected, but still generally showed significantly lower ratios of long-winged morphs when injected with metformin (Fig. 3*D*). This suggests that normal glucose metabolism is essential to the development of the long-winged morph, and interference with glucose production results in a shift to short winged morphs.

At this time, we have not been able to reliably measure glucose levels in nymphal hemolymph or tissues; therefore, the precise effects of metformin in developing nymphs remains unknown. What is striking is the effect metformin had on wing morph ratio—metformin caused a strong bias toward development of short-winged morphs in both males and females across treatments. Nymphal glucose levels thus also seem to be critical signals in wing morph determination.

Increased Concentrations of Dietary Glucose Result in Increased Transcription of the Insulin Receptors *NlInR1* and *NlInR2*. We also measured transcript expression in the insulin receptors *NlInR1* and *NlInR2* after glucose supplementation at increasing concentrations to rice seedlings. After 6 or 12 h of increased glucose supplementation to rice seedlings, there were no consistent or strong changes of expression of the insulin receptors *NlInR1* and *NlInR2* (*SI Appendix*, Fig. S1 *A–D*). However, by 24 h, there were significant increases in transcription of both *NlInR1* and *NlInR2* in brown planthoppers with higher levels of glucose supplementation (1, 5, or 15%) relative to low levels (0.2% or no extra glucose; *SI Appendix*, Fig. S1 *E* and *F*). This effect persisted at 48 h after glucose treatment, mainly for *NlInR2* (*SI Appendix*, Fig. S1 *G* and *H*). These results further demonstrate the connection between dietary glucose concentration and insulin pathway activation in the brown planthopper, although clearly changes in transcription are not a direct reflection of activation of the insulin signaling cascade, which would be expected to occur much more quickly than 24 h after increased glucose ingestion.

Knockdown of Insulin Signaling Pathway Genes Indicates Host Plant Glucose and Density Affect the Role of InR1 in Determining Long-Wing Morph. Previous studies have shown distinct roles for two insulin receptors (*InR1* and *InR2*) in wing dimorphism in brown planthoppers. RNAi-mediated down-regulation of *InR1* results in a shift to the short-winged morph, suggesting that *InR1* is necessary for long-wing morph development; down-regulation of *InR2* (a negative regulator of *InR1*) results in a shift to the long-winged morph, suggesting that *InR2* is necessary for short-winged morph development (37, 38). We tested whether increased glucose content changed the wing morph ratio when simultaneously down-regulating either of the two insulin receptors by RNAi (Fig. 4). Down-regulation of all of the genes was confirmed by qRT-PCR (*SI Appendix*, Fig. S2). Down-regulation of *InR1* led to a significant increase in the proportion of short-winged adults as expected, but this effect was dependent on density (two-way ANOVA, $P < 0.001$, df = 3). Specifically, injection of *InR1* double-stranded RNA (dsRNA) significantly increased the short-winged morph ratio, to 100%, at densities of 5 and 10 planthoppers per tube (Fig. 4) relative to *GFP* controls for both males and females (this was true for males at a density of one also, but the female nymphs reared at a density of one were already all short-winged). This agrees with previous reports on the effect of *InR1* knockdown on brown planthopper wing morph (37, 38). At densities of 20 per tube, there was no significant difference in ratios of short:long-winged morphs between *InR1* and *GFP* treatments, indicating that some density- dependent effects on wing polyphenism may operate independently of the insulin signaling pathway. At present, we hypothesize that density effects are transduced by a pathway other than insulin signaling, and that effects on wing morph are a result of the interplay of potentially conflicting signals from both the insulin and density signaling pathways (i.e., dietary glucose concentration vs. level of crowding).

The effects of *InR1* knockdown in brown planthoppers fed on glucose-supplemented rice seedlings were not the same as those fed on control rice seedlings (Fig. 4 *A–F*). When brown planthoppers feed on glucose-supplemented rice seedlings, this results in increased long-winged morphs. When *InR1* is decreased by RNAi, brown planthoppers have an increase in short-winged morphs. The combined experiment of feeding brown planthoppers on glucose-supplemented seedlings inhibited the effect of *InR1* knockdown and resulted in no differences in wing morph ratios between knockdowns and controls across all densities. This

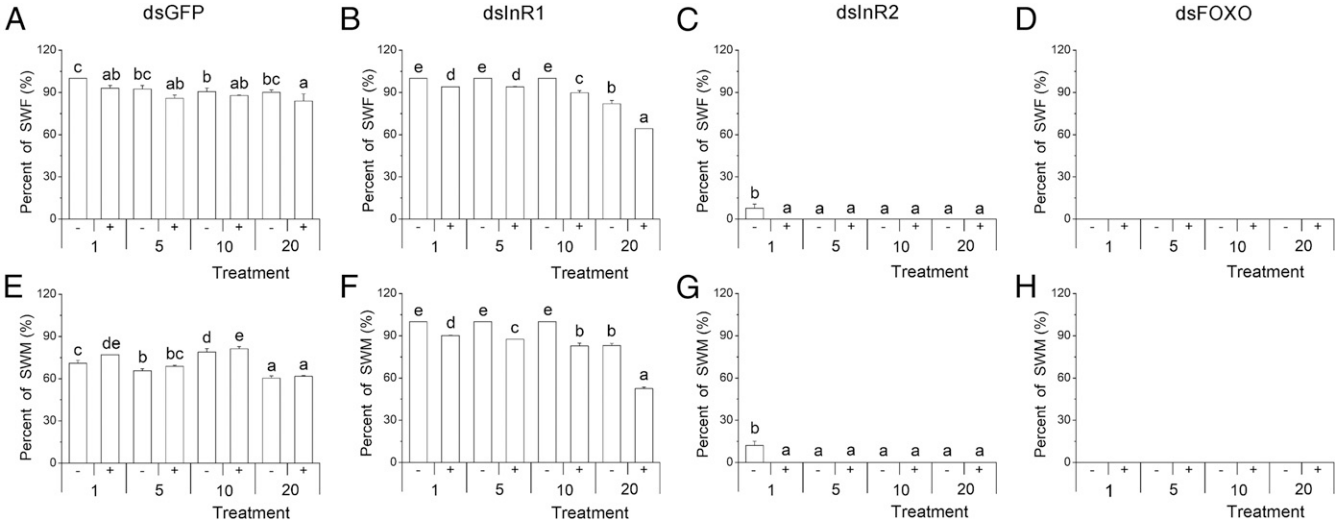

Fig. 4. Effect of RNAi-mediated knockdowns of *NlInR1*, *NlInR2*, and *NlFOXO*, glucose supplementation, and density on wing morph ratio. "+" indicates addition of a 1% glucose supplement to the rice seedling culture medium. "−" indicates no glucose was added. 1, 5, 10, and 20 indicate the number of brown planthoppers per tube (density). Two-way ANOVAs were performed, and Duncan's multiple-range test was used to differentiate between the means; different letters indicate $P < 0.05$. *A* and *E* show the percentage of short-winged morphs depends on glucose supplementation and density, but not on dsGFP injection. *B* and *F* show that knockdown with dsNlInR1 does not impact the ratio of short-winged to long-wing morphs more than glucose supplementation or density. *C* and *G* show that knockdown with dsNlInR2 results in an almost complete shift to the long-wing morph regardless of glucose level or density in males and females. *D* and *H* show that knockdown with dsFOXO results in 100% long-winged morphs in both males and females regardless of glucose concentration or density. (*A–D*) Female. (*E–H*) Male. Sample sizes for each experimental group are as follows: dsGFP− (n = 120/104/106/140 females, 118/128/104/96 males), dsGFP+ (n = 116/98/98/136 females, 78/160/106/188 males), dsInR1− (n = 120/132/152/59 females, 79/99/102/194 males), dsInR1+ (n = 98/100/136/168 females, 122/96/116/156 males), dsInR2− (n = 102/110/118/88 females, 82/124/126/140 males), dsInR2+ (n = 96/130/142/102 females, 126/88/126/142 males), dsFOXO− (n = 100/86/136/90 females, 122/144/122/138 males), dsFOXO+ (n = 94/124/114/130 females, 122/102/94/118 males). N/N/N/N indicates at densities of 1, 5, 10, and 20 per tube.

was true not only for females, but also for males. And at high densities (20 per tube), which were not affected by *InR1* knockdown in control plants, there was actually a significant increase in the proportion of long-winged adults compared with *GFP* controls on glucose-supplemented seedlings.

Unlike with *InR1*, density and plant glucose level had little effect on wing morph ratio after *InR2* knockdown (Fig. 4 *C* and *G*). Consistent with previous reports (38), knockdown of *InR2* caused a shift to long-winged morphs regardless of density, sex, or plant glucose level. In fact, *InR2* knockdown resulted in 100% long-winged adults, except at a density of one planthopper per tube on nonglucose-supplemented plants, although there was still a significant reduction in the proportion of short-winged adults in this treatment as well. Since increasing both density and glucose content, and knocking down *InR2*, all tend to increase the proportion of long-winged adults, this result was not unexpected.

It has previously been demonstrated that the effects of *InR1* and *InR2* on brown planthopper wing morph are due to negative regulation by the insulin-signaling cascade of the FOXO transcription factor (37, 38). Knockdowns of *FOXO* resulted in a strong shift to the long-winged morph, and this effect was dominant over knockdowns of all insulin signaling pathway genes (Fig. 4 *D* and *H*). We found that knockdown of *FOXO* through RNAi resulted in 100% long-winged adults, regardless of density, sex, or plant glucose level. As with *InR2*, *FOXO* knockdowns strongly favor development of long-winged morphs (Fig. 4 *D* and *H*). Increases in density and host glucose content would both tend to shift development to the long-winged morph, and since *FOXO* and *InR2* knockdown almost completely favor development of the long-winged morph, we did not expect to see interactions between these treatments. However, the effects of RNAi-mediated knockdown of *InR1* were impacted by variations in density and seedling glucose levels (Fig. 4).

In lower-glucose seedlings, *InR1* knockdown did result in an increased proportion of short-winged individuals at the lower densities, but not at the highest densities. And when nymphs were fed on the glucose-supplemented plants, there was no effect of knockdown of *InR1* (except at the highest density where nymphs were actually more likely to develop into long-winged morphs than *GFP* controls on high glucose plants). The fact that high densities seem to cause effects on wing morph independent of InR1 does not seem especially surprising. We had no a priori reason to suppose that whatever mechanism senses density in planthoppers, they operate wholly or even in part through the insulin signaling pathway. Other signaling pathways (e.g., JNK; ref. 36) may be better candidates as mechanistic determinants of wing polyphenism initiated through changes in density. However, the lack of effect of *InR1* knockdown on wing morph when planthoppers were fed on higher-glucose seedlings was more surprising. There is some possibility that this could reflect a pathway other than the insulin signaling pathway, or at least a receptor other than InR1, mediating the response to higher concentrations of glucose in host plants.

Conclusion

Our study is a step toward understanding the role that nutritional variation has in inducing condition-dependent, adaptive phenotypes. We have shown that the developmental response to both higher and lower glucose concentrations is most likely mediated through the insulin signaling pathway based on evidence to date (Fig. 5). It is important to note that interpretations of RNAi-induced phenotypes are complicated by the fact that RNAi represents a knockdown, and not knockout, of a particular gene product. Thus, there is some level of continued protein expression, and perhaps residual protein present, in dsRNA-treated study organisms. It is also important to consider our results in light of the possibility that higher plant glucose concentrations surpassed a threshold that allowed the limited quantities of InR1 in *InR1* knockdown individuals to activate the insulin signaling cascade, allowing enough repression of FOXO that development of some long-winged adults could occur (Fig. 5). Again, this is speculative at this point; however, we hypothesize that the insulin signaling pathway responds to increasing levels of glucose in a threshold-dependent manner under normal conditions, so that as glucose concentrations rise, circulating insulin-like peptides levels rise as well, more InR1 receptors are activated, and eventually FOXO suppression becomes great enough that development is switched to the long-winged morph (Fig. 5). Threshold-dependent responses could also account for the differential responsiveness of male and female brown planthoppers to inducers of long-wing morph development.

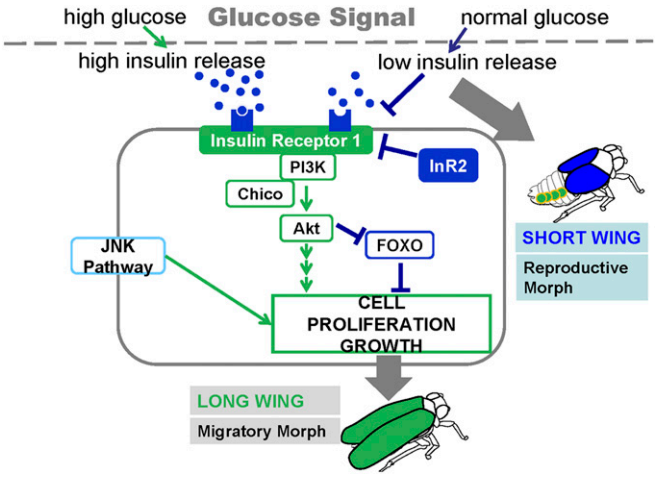

Fig. 5. Proposed model of the interaction between plant glucose concentration and wing polyphenism. High glucose concentrations in the phloem of rice plants result in release of higher amounts of insulin in the brown planthopper. Above a certain insulin threshold, activation of the insulin pathway is sufficient to repress the FOXO transcription factor and switch development to the long-wing, migratory morph. Lower plant glucose concentrations result in a subthreshold release of insulin, and FOXO is active, resulting in development of the short-winged, reproductive morph. Question marks indicate that ovary development was not investigated in this study and more work remains to be done to investigate the impact of the early glucose cue on lifetime fecundity in the brown planthopper.

In conclusion, this basic research into the physiological interaction between host plant and insect suggests that any variations in glucose concentrations in rice plants through selective breeding could alter brown planthopper wing-morph ratio in the field, with potential for insect pest management. More research remains to be done to fully explore the nutritional landscape that brown planthoppers face that lead to these adaptive phenotypes. The experimental results presented here provide a solid framework to further investigate how environmental signals are sensed and transduced into adaptive phenotypes. In addition, these results provide a starting point to investigate potential mechanistic linkages in the evolutionary tradeoff between dispersal and reproduction in wing polyphenic insects. At present, we know nothing of the mechanism(s) that account for reductions or delays in reproductive ability in the winged planthopper morph. Our results provide a strong framework to determine if similar signals and transduction pathways operate on the developing ovaries.

Materials and Methods

Insect and Rice Culture. The *Nilaparvata lugens* population was maintained in the X.L. laboratory and came originally from a culture provided by Z. R. Zhu (Institute of Insect Science, Zhejiang University, Hangzhou, China). The appropriate amount of rice culture media (nutrient solution) was added, and the rice was then cultured in an incubator (*SI Appendix*, Fig. S3). *N. lugens* were usually reared at densities of 6–10 animals per 100 cm^3 space (*SI Appendix*, Fig. S3). Adults were collected at different time points according to purpose of the experiment.

EVOLUTION

Cloning of *NlInR1*, *NlInR2*, and *NlFOXO* for dsRNA Synthesis. Total RNA was extracted, and first-strand cDNA was transcribed. The *InR1*, *InR2*, and *FOXO* DNA fragment used as templates for dsRNA synthesis were amplified by PCR using Ex-Taq polymerase (Takara). The DNA fragments were then cloned into pMD-18T vector (Takara).

dsRNA Preparation and Injection. dsRNA was synthesized using a kit from Promega. The primers for amplifying the DNA fragments for dsRNA synthesis of *NlInR1*, *NlInR2*, and *NlFOXO* and *GFP* are listed in *SI Appendix*, Table S1. Fourth instar *N. lugens* nymphs were used for dsRNA injection.

qRT-PCR. qRT-PCR was carried out using a kit from Roche (Roche Applied Science). Reverse transcription was carried out as described by the supplier (Roche Applied Science). The primers used are shown in *SI Appendix*, Table S2 (including product size and amplification efficiency).

Glucose Concentration Assay. The glucose concentration in rice was measured using a Glucose (HK) Assay Kit (GAHK-50; Sigma). The rice stalks were used for glucose measurement. Rice plants of different developmental stages were collected from the greenhouse of the China Rice Research Institute and fields in Hainan Province, China (E109.475084, N18.304290). Six developmental stages from the greenhouse and three development stages from the field were collected.

Injection of Metformin and Glucose. Metformin (Sangon Biotech) was dissolved into distilled water at 0.2 mmol/L. For glucose injection, a 0.3 mM glucose solution was prepared (in water) and autoclaved. The injection procedure for glucose and metformin was the same. A volume of 0.2-μL solution per fourth instar nymph was injected. Water was used as a control. The injection procedures were identical to those for dsRNA injection described above.

Statistical Analysis. All statistical analyses were carried out using SPSS 20.0. Student's *t* test was used to compare changes in transcript abundance after dsRNA treatments. Duncan's multiple range test was used to compare the transcriptional changes of *InR1* and *InR2* after treatment with different concentrations of glucose. For statistical analysis of the change in wing morph ratio between control and experimental individuals, we used an interactive two-way ANOVA (analysis of variance). Duncan's multiple range test was used for statistically significant differences of the main effect or interaction effects of the two-way ANOVA.

See *SI Appendix, SI Materials and Methods* for more details on the methods we used.

ACKNOWLEDGMENTS. This research was supported by National Natural Science Foundation of China Grants 31672023, 31471771, and 31741107 (to X.L.), National Science Foundation Grant IOS-0919730, and National Institute of Food and Agriculture, Department of Agriculture Accession No. 1001738 (to L.C.L.).

1. Price TD, Qvarnström A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proc Biol Sci* 270:1433–1440.
2. Brisson JA (2010) Aphid wing dimorphisms: Linking environmental and genetic control of trait variation. *Philos Trans R Soc Lond B Biol Sci* 365:605–616.
3. Ishikawa A, Gotoh H, Abe T, Miura T (2013) Juvenile hormone titer and wing-morph differentiation in the vetch aphid Megoura crassicauda. *J Insect Physiol* 59:444–449.
4. Ishikawa A, Miura T (2009) Differential regulations of wing and ovarian development and heterochronic changes of embryogenesis between morphs in wing polyphenism of the vetch aphid. *Evol Dev* 11:680–688.
5. Ishikawa A, Miura T (2013) Transduction of high-density signals across generations in aphid wing polyphenism. *Physiol Entomol* 38:150–156.
6. Zera AJ (1984) Differences in survivorship, development rate and fertility between the longwinged and wingless morphs of the waterstrider, *Limnoporus canaliculatus*. *Evolution* 38:1023–1032.
7. Zera AJ, Brisson JA (2015) Induction and function of polyphenic morphs: Proximate regulatory mechanisms and evolutionary implications. *Integrative Organismal Biology*, eds Martin LB, Ghalambor CK, Woods HA (Wiley, Hoboken, NJ).
8. Zera AJ, Denno RF (1997) Physiology and ecology of dispersal polymorphism in insects. *Annu Rev Entomol* 42:207–230.
9. Zera AJ, Harshman LG (2003) The physiology of life history trade offs in animals. *Annu Rev Ecol Syst* 32:95–126.
10. Ishikawa A, et al. (2012) Screening of upregulated genes induced by high density in the vetch aphid Megoura crassicauda. *J Exp Zool A Ecol Genet Physiol* 317:194–203.
11. Braendle C, Davis GK, Brisson JA, Stern DL (2006) Wing dimorphism in aphids. *Heredity (Edinb)* 97:192–199.
12. Ishikawa A, et al. (2012) Screening of upregulated genes induced by high density in the vetch aphid Megoura crassicauda. *J Exp Zool A Ecol Genet Physiol* 317:194–203.
13. Zera AJ, Potts J, Kobus K (1998) The physiology of life-history trade-offs: Experimental analysis of a hormonally induced life-history trade-off in Gryllus assimilis. *Am Nat* 152: 7–23.
14. Boggs CL (1992) Resource allocation: Exploring connections between foraging and life history. *Funct Ecol* 6:508–518.
15. Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. *Funct Ecol* 23:27–37.
16. Clark RM, Zera AJ, Behmer ST (2015) Nutritional physiology of life-history trade-offs: How food protein-carbohydrate content influences life-history traits in the wing-polymorphic cricket Gryllus firmus. *J Exp Biol* 218:298–308.
17. Jaenike J (1990) Host specialization in phytophagous insects. *Annu Rev Ecol Syst* 21: 243–273.
18. van Noordwijk A, de Jong G (1986) Acquisition and allocation of resources: Their influence on variation in life-history tactics. *Am Nat* 128:137–142.
19. Zera AJ (2003) The endocrine regulation of wing polymorphism in insects: State of the art, recent surprises, and future directions. *Integr Comp Biol* 43:607–616.
20. Denno RF, Roderick GK (1990) Population biology of planthoppers. *Annu Rev Entomol* 35:489–520.
21. Kisimoto R (1965) Studies on polymorphism and its role playing in the population growth of brown planthopper, Nilaparvata lugens (Stal). *Bull Shikoku Agric Exp Stn* 13:101–106.
22. Denno RF, Roderick GK, Olmstead KL, Dobel HG (1991) Density-related migration in planthoppers (Homoptera: Delphacidae): The role of habitat persistence. *Am Nat* 138: 1513–1541.
23. Simpson SJ, Sword GA, Lo N (2011) Polyphenism in insects. *Curr Biol* 21:R738–R749.
24. Zhang ZQ (1983) A study on the development of wing dimorphism in the rice brown planthopper, Nilaparvara lugens Stål. *Acta Entomol Sin* 26:260–267.
25. Li JL, et al. (2013) Effects of high temperature on rice source-sink characteristics during heading stage to grain filling stage. *Zhonghua Nongye Qixiang* 34:23–29.
26. Jeffrey MH (1982) A possible correlation between light trap catches of the brown planthopper Nilaparvata lugens (Stål) and the stage of development of the rice crop [Bangladesh, India, Malaysia, the Philippines, Taiwan]. Miscellaneous Reports - Centre for Overseas Pest Research (United Kingdom), no. 57.
27. Padgham D (1983) The influence of host-plant on the development of the adult brown planthopper, Nilaparvata lugens (Stål) (Hemiptera: Delphacidae), and its significance in migration. *Bull Entomol Res* 73:117–128.
28. Riley J, Reynolds D, Farrow R (1987) The migration of Nilaparvata lugens (Stål) (Delphacidae) and other Hemiptera associated with rice during the dry season in the Philippines: A study using radar, visual observations, aerial netting and ground trapping. *Bull Entomol Res* 77:145–169.
29. Dingle H (1982) Function of migration in the seasonal synchronization of insects. *Entomol Exp Appl* 31:36–48.
30. Avonce N, Leyman B, Thevelein J, Iturriaga G (2005) Trehalose metabolism and glucose sensing in plants. *Biochem Soc Trans* 33:276–279.
31. Ayoade O, Morooka S, Tojo S (1999) Enhancement of short wing formation and ovarian growth in the genetically defined macropterous strain of the brown planthopper, Nilaparvata lugens. *J Insect Physiol* 45:93–100.
32. Bertuso AG, Morooka S, Tojo S (2002) Sensitive periods for wing development and precocious metamorphosis after precocene treatment of the brown planthopper, Nilaparvata lugens. *J Insect Physiol* 48:221–229.
33. Kisimoto R (1956) Effect of crowding during the larval period on the determination of the wing-form of an adult plant-hopper. *Nature* 178:641–642.
34. Iwanaga K, Tojo S (1986) Effects of juvenile hormone and rearing density on wing dimorphism and oöcyte development in the brown planthopper, Nilaparvata lugens. *J Insect Physiol* 32:585–590.
35. Wang XR, Zhang CD (1981) Studies on factors of wing dimorphism of brown planthopper Nilaparvata lugens (Stål). *Entomol Knowl* 18:4.
36. Lin X, et al. (2016) JNK signaling mediates wing form polymorphism in brown planthoppers (Nilaparvata lugens). *Insect Biochem Mol Biol* 73:55–61.
37. Lin X, Yao Y, Wang B, Emlen DJ, Lavine LC (2016) Ecological trade-offs between migration and reproduction are mediated by the nutrition-sensitive insulin-signaling pathway. *Int J Biol Sci* 12:607–616.
38. Xu HJ, et al. (2015) Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* 519:464–467.
39. Lin X, Yao Y, Wang B, Lavine MD, Lavine LC (2016) FOXO links wing form polyphenism and wound healing in the brown planthopper, Nilaparvata lugens. *Insect Biochem Mol Biol* 70:24–31.
40. Boussageon R, et al. (2012) Reappraisal of metformin efficacy in the treatment of type 2 diabetes: A meta-analysis of randomised controlled trials. *PLoS Med* 9:e1001204.
41. Kirpichnikov D, McFarlane SI, Sowers JR (2002) Metformin: An update. *Ann Intern Med* 137:25–33.
42. Viollet B, et al. (2012) Cellular and molecular mechanisms of metformin: An overview. *Clin Sci (Lond)* 122:253–270.