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Reversible Polyphenism in *Trissolcus japonicus* (Hymenoptera: Platygastridae) Induced by Stink Bug Egg Size[1]

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Polyphenism occurs when several distinct phenotypes are produced as a result of changing environmental conditions. In most cases, the phenotype is reversible from one generation to the next, unless a natural or artificial genetic selection occurs and the new morphological characteristic becomes fixed and passed on to the next generations when there is a selective advantage offered by the new trait. Polyphenism is one of the main reasons for the remarkable success of insects existing in continuously changing environments. This adaptation can lead to a great diversity of morphological forms. Several groups of eusocial insects such as honey bees, ants, and termites have been the focus of considerable research to elucidate the clues that trigger this phenomenon (Kamakura 2011, Nature 473: 478–483; Cornette et al. 2008, J. Insect Physiol. 54: 922–930; Abouheif and Wray 2002, Science 297: 249–252). Other insect groups that have been intensively studied include moths and butterflies (Lepidoptera), beetles (Coleoptera: Lucanidae, Scarabaeidae), aphids (Homoptera), and grasshoppers (Orthoptera) (Nijhout 1999, BioScience 49: 181–192; Pener and Simpson 2009, Adv. Insect Physiol. 36: 1–286; Emlen 1994, Proc. R. Soc. B. 256: 131–136). Environmental conditions that trigger shifts in phenotype include nutrient quantity and quality, temperature, humidity, population density, day length, and light intensity, among others. Some of these environmental factors interacting with hormonal levels (e.g., ecdysone and juvenile hormone) present in the insect body induce phenotypic changes that are better adapted to new environmental conditions (Ma et al. 2011, Proc. Natl. Acad. Sci. USA 108: 3882–3887; Anstey et al. 2009, Science 323: 627–630; Matsuura et al. 2010, Proc. Natl. Acad. Sci. USA 107: 12963–12968; Emlen and Nijhout 1999, J. Insect Physiol. 45: 45–53). Simpson et al. (2011, Curr. Biol. 21 R738–R749) reviewed research that has been conducted on some of the best known examples

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of polyphenism in insects, and Nijhout (1999) discussed in detail the control mechanisms of polyphenic development in insects. Contrary to the extensive research that has been conducted with polyphenic expressions in social hymenopterans, there is a lack of information on polyphenism in Parasitica (Hymenoptera: Apocrita) insects.

In this study, we describe a unique phenotypic adaptation of the egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Platygastridae) induced by the egg size of three stink bug hosts, brown marmorated stink bug, *Halyomorpha halys* (Stål); redshouldered stink bug, *Thyanta custator* (F.), and spined stink bug, *Loxa flavicollis* (Drury). In October 2011 and February 2012, adult specimens of *H. halys* and *T. japonicus*, respectively, were received in the Florida Biological Control Laboratory quarantine facility in Gainesville from the USDA–Agricultural Research Service Beneficial Insect Introduction Research Unit in Newark, DE, to be used in host specificity tests with *H. halys* and stink bugs collected in Florida. Recently emerged and mated *T. japonicus* females were exposed during 24 h to 1-d-old egg masses of *H. halys*, *T. custator*, and *L. flavicollis* in a laboratory maintained at 26°C, 16-h photoperiod (16:8 h light:dark), and 50–55% relative humidity. One replication consisted of a single *T. japonicus* female, sexed using a microscope and placed in a 37-ml (10 dram) snap-cap vial with one individual egg mass of the stink bug tested. Each egg mass was attached to double-sided tape (1.27×1.0 cm) and taped to white card stock (5.75×0.34 cm) (Hammermill, item no. 08640-0, Memphis, TN) and placed into the vial. A completely randomized design with 20 replications was used.

Parasitoid emergence from the egg masses occurred in 10 to 12 d under the environmental conditions used. At the end of the experiment, vials were inspected and the numbers of parasitoids emerged; their length, width, weight, sex; the numbers of unhatched eggs; and stink bug nymph emergence were recorded. The length and width of the parasitoids were measured using a SS Micro Ruler (Electron Microscopy Sciences, Cat. no. 62096-08, Hatfield, PA). The body weight was recorded using an analytical balance (Denver Instrument, SI-234, Bohemia, NY). Data were subjected to an analysis of variance (SAS Institute, Cary, NC), and sample means were separated using a least significant difference (LSD) procedure, when appropriate. Standard deviations were determined for all means. Results indicated a significant effect of stink bug egg size ($F = 6.01$; df $= 2$; $P = 0.01$; LSD Test) on the resulting phenotype (length, width, weight) of *T. japonicus* adults (Table 1; Fig. 1). It is suspected that the amount of nutrient present in the differently sized eggs triggered the polyphenic response in the parasitoid. When first-generation adult wasps of the two alternative phenotypes were exposed to eggs of the original natural host *H. halys*, the new polymorphic character (smaller or larger body size) was reversible, returning to the standard size of the original natural host. Numerous research studies conducted with other insect groups indicated an adaptive response to diverse changing environmental conditions such as nutrient level (Bazazi et al. 2011, Proc. R. Soc. B 278: 356–363; Emlen and Nijhout 1999).

In this study, the alternative phenotypes that were expressed (smaller or larger adult egg-parasitoids) were likely triggered by the amount of nutrients that the parasitoid larvae encountered during their development inside a relatively large egg (*L. flavicollis* egg size: 1.6 to 1.7 mm diam; 2.2 to 2.4 mm height), standard or middle egg size from the egg parasitoid natural host *H. halys* in China (*H. halys* egg

Table 1. Number emerged and length, width, and weight of adult polymorph parasitoids from different egg sizes of three stink bug species.

Stink Bug Species	No. of ♀ Parasitoids Emerged	No. of ♂ Parasitoids Emerged	No. of Eggs Unhatched	Parasitoid Length (mm)	Parasitoid Width (mm)	Parasitoid Weight (mg)
Loxa flavicollis	4.3 ± 1.0 a*	1.1 ± 0.6 a	0 a	1.68 ± 0.16 a	0.69 ± 0.04 a	0.0025 ± 0.0003 a
Halyomorpha halys	23.4 ± 0.71 b	1.9 ± 1.41 a	1.0 ± 1.0 a	1.35 ± 0.14 b	0.61 ± 0.03 b	0.0018 ± 0.0002 b
Thyanta custator	6.1 ± 2.3 a	2.0 ± 1.0 a	12.6 ± 4.1 b	0.92 ± 0.07 c	0.33 ± 0.05 c	0.0009 ± 0.0003 c

* Means ± SD within a column followed by the same letter are not significantly different ($P < 0.05$; LSD).

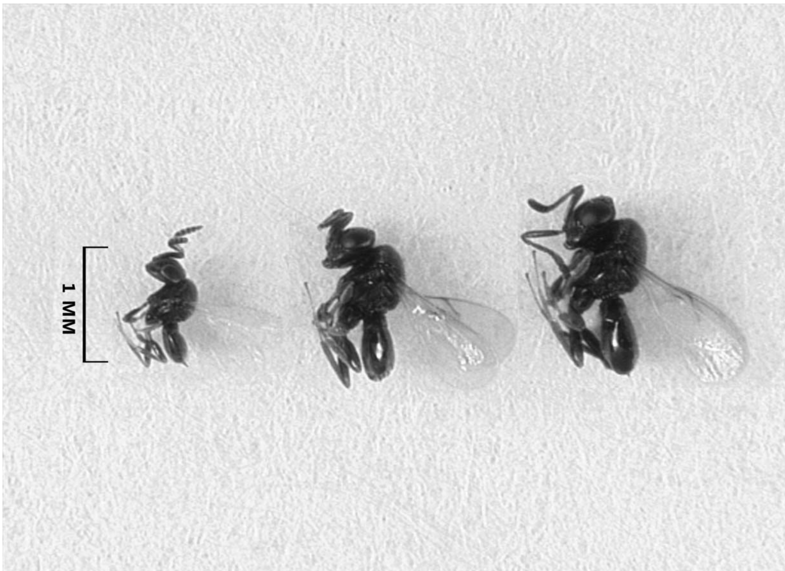

Fig. 1. ***Trissolcus japonicus*** **(Hymenoptera: Platygastridae) polyphenism forms induced by egg size of the stink bugs** ***Thyanta custator*** **(left),** ***Halyomorpha halys*** **(middle), and** ***Loxa flavicollis*** **(right) (Heteroptera: Pentatomidae).**

size: 1.2 to 1.3 mm diam; 1.6 to 1.8 mm height), and smaller egg size (*T. custator* egg size: 0.6 to 0.7 mm diam; 0.8 to 0.9 mm height). From an evolutionary perspective this could be of practical significance in determining whether the modified phenotypes will have improved fitness in a continuously changing environment above the original phenotype obtained from *H. halys*, its natural host in China.

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