

Biological Journal of the Linnean Society, 2016, ••, ••-••. With 4 figures.

Multiple lines of anti-predator defence in the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae)

CHANGKU KANG¹, HYUNGMIN MOON², THOMAS N. SHERRATT¹, SANG-IM LEE^{2,3} and PIOTR G. JABLONSKI^{2,4}*

¹Department of Biology, Carleton University, Ottawa, ON, Canada ON K1S 5B6

²School of Biological Sciences, Seoul National University, 08826, 1 Gwanak-ro, Seoul, South Korea ³Institute of Advanced Machinery and Design, Seoul National University, 08826, 1 Gwanak-ro, Seoul, South Korea

⁴Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679, Warsaw, Poland

Received 4 May 2016; revised 26 May 2016; accepted for publication 30 May 2016

Many species have evolved a suite of anti-predator defences, rather than a single defence. These multiple defences may operate in synchrony or separately at different stages of predation sequence to protect the prev. However, empirical documentation on how multiple defences, as a whole, combine to protect prey, as well as quantitative evaluations of how and when they are deployed, are scarce. In the present study, we investigated the univoltine spotted lanternfly, Lycorma delicatula, which has cryptic forewings, defensive chemicals, and multiple behavioural defences, including rapid jumping away, sudden display of its conspicuous hindwings and abdomen (a startle/ deimatic display), and death feigning. The aims of the present study were to: (1) characterize the modality of sensory stimuli that trigger the behavioural defences; (2) identify the stage(s) of the predation sequence in which L. delicatula employs each behavioural defence; and (3) investigate a range of intrinsic/extrinsic factors that might affect the execution of anti-predator responses. First, a preliminary test that simulated a range of sensory stimuli on L. delicatula suggested that they rarely responded to nontactile stimuli. This suggests that the species relies on crypsis as a primary defence unless it is physically contacted. Next, we simulated predatory attacks on the species at two different times of year (early and late season as adults). When physically contacted, the primary response of individuals was jumping away. However, when jumping was initially hindered (by grabbing), they then tended to employ deimatic display. Intriguingly, we found clear seasonal differences in these post-attack defences: after performing deimatic display, individuals were more likely to jump away in the early season, whereas death feigning was more frequent in the late season. We present adaptive explanations for this seasonal switch in anti-predator responses. © 2016 The Linnean Society of London, Biological Journal of the Linnean Society, 2016, 00, 000–000.

KEYWORDS: animal coloration – crypsis – death feigning – deimatic display – multiple defences – secondary defence – startle display.

INTRODUCTION

A predator-prey interaction comprises of a number of sequential events, including prey detection, pursuit, seizing, handling, and consumption (Edmunds, 1974; Bateman, Vos & Anholt, 2014). Reflecting this hierarchy, individual prey species have evolved a range of anti-predator adaptations that operate at different stages of the predation sequence (Endler, 1991; Caro, 2005). For example, one of the most common forms of prey defence is a protective coloration, notably camouflage, aposematism or mimicry (Cott, 1940; Ruxton, Sherratt & Speed, 2004). These protective appearances usually operate as a first line of defence and protect the prey in the early stages of predation sequence by preventing detection or pursuit by predators (Ruxton *et al.*, 2004). Nevertheless, many prey species have also evolved secondary/tertiary lines of defence, a defensive portfolio (Briton, Planqué & Franks, 2007), with these operating once their primary defence has failed (Edmunds, 1974; Brown, Boettner & Yack, 2007). To date, the secondary defences of prey have been usually studied in

^{*}Corresponding author. E-mail: piotrjab@behecolpiotrsangim.org

isolation (Edmunds, 1974; Ruxton *et al.*, 2004). However, to fully understand how prey defences protect prey, it is crucial to consider the prey's defences as a whole and how they are strategically deployed to deter predators.

Multiple anti-predator adaptations of a species can evolve in circumstances where (1) they are preyed upon by multiple predator species that use different sensory cues to detect the prey so that a defence in one sensory modality does not provide protection against another predator that uses a different sense (Silveira, Oliveira & Trigo, 2010) and/or when (2) primary defences are prone to failure, perhaps as a result of some form of constraint on their effectiveness (Endler, 1991; Caro, 2005; Briton *et al.*, 2007). To understand the selection pressure that shaped the multiple defences in a species, it is important to examine how and when prey execute their multiple anti-predator defences against predatory threats throughout the predation sequence.

Among the secondary defences, there is a growing interest in deimatic display (otherwise called startle or frightening display) (Umbers, Lehtonen & Mappes, 2015). Deimatic display is a behaviour in which otherwise cryptic prey suddenly display previously hidden conspicuous body parts in response to the approach or attack of a predator (Umbers et al., 2015). Typical examples of deimatic display can be found in moth genus *Catocala*, which have bark-like cryptic forewings and conspicuous hindwings that are displayed when disturbed (Schlenoff, 1985). Deimatic display is usually employed by palatable species as a deceptive signal, although it is also observed in chemically defended prey as an honest signal of unpalatability (Edmunds, 1974; Lenzi-Mattos et al., 2005; Umbers & Mappes, 2015).

Deimatic display can be adaptive only if either (1)the attacked predator is frightened off and leaves the vicinity or (2) the prey escapes away successfully when the predator is startled (Edmunds, 1974). Empirical evidence to date supports the former hypothesis, in that avian predators were shown to be startled by conspicuous displays and they occasionally attempt to flee (Vallin et al., 2005; Olofsson et al., 2012). However, we know little about the prey's escape decision. Escaping is likely to incur both energetic and opportunity costs (Lima & Dill, 1990). It also increases the chance of being detected if the prey had not already been seen (Broom & Ruxton, 2005). Therefore, the escape decision is context dependent and influenced by a range of trade-offs (Ydenberg & Dill, 1986). In the case of the prey with deimatic display, escaping away after the startle display would be beneficial only when follow-up predatory attacks are likely to occur (e.g. the signal is bluff), whereas staying put would likely be selected

for if predators are generally dissuaded by the display (e.g. the display is an honest signal of unpalatability or mimicking intimidating signals such as eyespots). Therefore, quantitative evaluation of the prey escape decision during deimatic display may shed light on its adaptive significance.

Death feigning is another form of secondary defence widespread in many taxa (Rogers & Simpson, 2014), although the term may be something of a misnomer because it extends to instances in which death is not mimicked (Romanes & Darwin, 1883; Rogers & Simpson, 2014). As an umbrella term, death feigning may provide different benefits under different conditions, including (1) the increased chance of escape from motion-oriented predators (e.g. when the predator pursues other nearby prey); (2)the increased chance of successful mating with cannibalistic partner; and (3) making the prey body difficult to consume through a rigid posture (Thompson et al., 1981; Honma, Oku & Nishida, 2006; Bilde et al. 2006). A common feature of death feigning is that it is usually induced by a physical contact. which suggests that death feigning has evolved as a post-attack (i.e. after a physical contact) defence in most prey species (Miyatake et al., 2004; Rogers & Simpson, 2014).

Throughout its life cycle, an individual is often exposed to seasonal change. This change frequently involves an alteration in resource availability and/or predation risk, and animals often compensate for these changes through behavioural modifications (Wilson & Boelkins, 1970; Beck et al., 2007; Mappes et al., 2014). To date, there have been only a few attempts to explore the seasonal variation in antipredator responses (Buitron, 1983; Hileman & Brodie, 1994; Mappes et al., 2014). Seasonal variation in anti-predator responses can occur because a predatory threat imposes different fitness consequences on different stages of a prey's life cycle (Buitron, 1983) and/or because the efficacies of anti-predator responses depend on the timing of a season (Hileman & Brodie, 1994; Mappes et al., 2014).

Our study species, the spotted lanternfly, Lycorma delicatula (White, 1845) (Hemiptera: Fulgoridae), is a univoltine planthopper native to China and South Asia, although it has recently invaded other nations such as South Korea and the United States (Han et al., 2008; Dara, Barringer & Arthurs, 2015). This insect undergoes a drastic morphological change from its last instar to adult: fully-formed wings appear and the abdomen colour changes from black/ red patterns with white spots to yellow/black stripes. Adult L. delicatula has evolved multiple anti-predator defences, including the cryptic forewing colour that resembles that of its host tree Ailanthus altisdefensive chemicals, and a number sima, of



Figure 1. Photos of *Lycorma delicatula* in normal resting status (a female in the middle and two males) (A), during deimatic display (B), and during death feigning (C).

behavioural defences, such as rapid escape jumping, deimatic display that suddenly reveals its contrasting hindwings and yellow/black banded abdomen, and death feigning (Xue & Yuan, 1996; Kang, Lee & Jablonski, 2011) (Fig. 1; see also Supporting information, Videos S1–S3).

In the present study, we simulated various predatory approaches/attacks and identified the performance of anti-predator defences through a predation sequence in L. delicatula and the intrinsic/extrinsic factors that affect the performance of anti-predator responses.

MATERIAL AND METHODS

ANTI-PREDATOR RESPONSES TO THE SIMULATED PHYSICAL ATTACKS

Preliminary testing on adult L. delicatula indicated that this insect rarely responded to nontactile stimuli (i.e. in pre-attack stages; see Supporting information, Fig. S1). We therefore investigated the anti-predator responses of L. delicatula in the postattack stage by stimulating wild-caught specimens in a tactile manner (i.e. post-attack). This experiment was conducted in Mt Gwan-ak, Seoul, South Korea $(N37.427^{\circ}, E126.916^{\circ})$ at two different times of year. The invaded Korean populations are genetically identical to those from their origins (Kim et al., 2013), and so we tentatively considered the behaviour of these individuals to be representative of the behaviour of the species as a whole. We conducted the early season trials between 18 and 21 August and the late season trials between 15 and 23 October 2010. The early season represents the early adulthood of L. delicatula when almost all nymphs have completed the final moult to adults, whereas the late season represents the late adulthood of L. delicatula near the end of their life cycle (Kim et al., 2011). First, we collected adult *L. delicatula* exclusively from A. altissima trees and kept them in insect containers with plucked branches and leaves. We then subsequently tested each individual within 30 min of capture using the procedures outlined below.

We assumed that major predatory threats to adult L. delicatula are from birds (Kang et al., 2011) and thus simulated two types of predatory attacks in sequence: pecking and grabbing. Pecking was performed by placing each insect on a branch of A. altissima (radius 3 cm), waiting until the insect had settled on the branch, approaching perpendicularly to the insect's forewings, and then gently pecking it with tweezers. We note here that the pecking stimulus did not damage their wings or bodies. Grabbing was performed after observing the response of the insect to the pecking stimulus to the same individual. Here, the experimenter grabbed the insect laterally with their fingers, lifted it, gently squeezed its body for less than 1 s, and dropped it on the ground from a height of approximately 10 cm. Then, we observed and recorded the anti-predator response of each insect. In this way, we simulated a situation in which prey are not immediately consumed by a predator but, instead, have an opportunity to execute behavioural defences after being seized. This situation is not unrealistic, given that birds usually need to handle L. delicatula before consuming, during which time this insect had a chance to execute its post-attack defences (C. Kang, pers. observ.: a domestic chick attacking L. delicatula).

For each type of stimulation, we recorded (1) whether the bug stayed put or jumped away; (2) when the insect stayed put, whether it performed deimatic display, a death feigning display or adopted no display at all; and (3) when the bug displayed, how and when were the displays terminated. The end point of each observation was therefore either (1) the insect jumped away without display or (2) stayed put without any active defence, or (3) it terminated its deimatic or death feigning display by closing its wings or jumping away. We note here that,

during death feigning, the insects retained deimatic display (wing display) (Fig. 1B, C) and so death feigning could be considered as an additional defence of individuals already performing deimatic display. Deimatic display with death feigning (hereafter simply termed 'death feigning') was clearly discriminable from deimatic display without death feigning in that the insect collapsed on the ground with all legs folded into the abdomen without any movements during death feigning (Fig. 1C; see also Supporting information, Video S3), whereas it supported its body with legs during deimatic display (Fig. 1B; see Supporting information, Video S1). Only one experimenter (CK) performed all stimulations to induce similar pecking/grabbing stimuli between individuals. Consistent with our earlier findings (see 'response to various non-tactile sensory stimuli' section in Supplementary materials), no tested insect showed any responses in response to the approach of the tweezers, although (as noted in our observations) many did flee as the tweezers touched the forewings.

A potentially important factor influencing the decision to escape or not is the probability of successful escape (Ydenberg & Dill, 1986). In L. delicatula, this probability may be determined by how quickly it can jump away in response to the attack (i.e. latency from the moment of attack to escape). If it is sufficiently quick and agile to escape from the predator that has made an attack (i.e. the reaction latency is low), then the chance of avoiding the predator altogether would be high. Clearly, however, if it reacts slowly (i.e. the reaction latency is high), then the risk of being seized by the predator, which can lead to a fatal injury of the prey, would be high. Therefore, we predicted an intraspecific relationship between escape ability and escape decision (Ohno & Miyatake, 2007). Thus, we expected that individuals with a short reaction latency might be more likely to jump away in response to a tactile stimulus, whereas individuals with a long reaction latency would rely more on alternative defences such as deimatic display. To explore this possibility, we first measured the latency of response of individuals in the pecking experiment from the initial moment of a tactile stimulation to jumping away by recording the behaviour of each insect using a high-speed camera (1000 fps; Troubleshooter 1000 ME; Fastec Imaging). Because the relationship can hold only if the latency is a repeatable trait within individuals, we measured the reaction latency twice for 22 individuals. For the same reason, we measured the duration of deimatic display twice for 15 individuals by grabbing the individuals again following the previous grabbing stimulus.

Once the testing was completed, we carefully inspected the presence of wing damage on the specimen's wings as an indirect indication of unsuccessful past predatory attempts (Shapiro, 1974; Kang *et al.*, 2011). A total of 98 adults (49 males and 49 females) were tested in the early season, whereas 91 adults (45 males and 46 females) were tested in the late season. We adhered to the ethical guidelines of the Association for the Study of Animal Behaviour (Animal Behavior Society, 2012) and our experimental procedures involved non-invasive techniques. However, *L. delicatula* was considered as an invasive national pest in local areas and so, once the testing was completed, we froze them to avoid recapturing of the same individuals rather than re-locating the tested individuals. No permission was required for capturing and freezing them at the time of testing.

STATISTICAL ANALYSIS

To compare the frequencies of each behavioural response of the lanternflies to pecking and grabbing between season and sex, we used a Cochran–Mantel– Haenszel chi-squared test, with the null hypothesis that the behavioural responses did not differ at the population level between seasons or sexes.

On inspecting our data on the responses of lanternflies to grabbing, we found substantial interindividual variation with respect to (1) whether L. delicatula jumped away during deimatic display; (2) the duration of deimatic display; and (3) whether L. delicatula performed death feigning or not. To identify the factors that affected the execution of each response, we employed path analysis (Loehlin, 2011). One of the advantages of path analysis over multiple regression is that it can specify relationships between both dependent (called endogenous variable in path analysis) and independent (exogenous) variables, allowing a test of the fit of the complex relationships in one analysis, thus reducing type I error (Loehlin, 2011). In the path analysis, sex and season were treated as exogenous variables that were independent from other variables, with weight considered as an endogenous variable that was affected by both sex and season. We considered three types of anti-predator responses as endogenous variables that are affected by the above three factors; whether the insect jumped away during deimatic display (binary variable), the duration of deimatic display (continuous), and whether the insect feigned death or not (binary). Because we had no prior expectation of the nature of the relationships, we considered all combinations of exogenous and endogenous variables as the null relationship (see Supporting information, Fig. S2). We additionally anticipated a relationship in which death feigning has an effect on the duration of deimatic display because L. delicatula always maintained deimatic display during death feigning. Binary variables were

coded as either 0 or 1 sensu Rosseel (2012) (male = 0, female = 1; early season = 0, late season = 1; death feigning N = 0, Y = 1; escaping during deimatic display N = 0, Y = 1). The duration of the deimatic display was log-transformed to meet the normality assumptions in all analysis. Because all death feigners displayed (but not the reverse), the duration of this behaviour was not used in the above analyses because the two measures were highly collinear (Pearson correlation coefficient r = 0.94).

To confirm the repeatability of both reaction latency and deimatic display duration within individuals, we estimated the intra-class correlation (ICC) coefficient, testing the null hypothesis that the ICC is 0 at population level. A rejection of the null hypothesis would mean that within individual consistency likely exists, such that the trait exhibits among-individual level variation. We also tested whether wing damage had accumulated during a season by fitting generalized linear models (GLZ) with the presence/absence of wing damage in individuals as a binary response variable, with season and sex as explanatory variables. All statistical analyses were two-tailed and conducted in R, version 3.1.2 (R Core Team, 2014).

RESULTS

We found no significant differences between sex (Cochran-Mantel-Haenszel chi-squared test: $M^2 = 1.78$, d.f. = 2, P = 0.41) (Fig. 2A) and season $(M^2 = 1.71, d.f. = 2, P = 0.42)$ on the frequency of the insects' responses to pecking (in which they either jumped away, displayed deimatic signals and closed wings, or stayed without any active defence) (Fig. 2A). A majority of the individuals jumped away in both early (92%; 90 out of 98 individuals) (Fig. 2A) and late season (92%; 84 out of 91 individuals). However, when the insects were seized in the subsequent grabbing assay, we found significant differences in the frequency of overall responses (in which they first exhibited a deimatic display and then closed their wings, a deimatic display before jumping away, a death feign ending with closing wings, or stayed without any active defence) between sex (Cochran-Mantel-Haenszel chi-squared test: $M^2 = 14.51$, d.f. = 3, P = 0.002) (Fig. 2B) and season ($M^2 = 51.30$, d.f. = 3, P < 0.001). The sexual difference was observed mainly because some males did not execute a deimatic display (13% of total), whereas all females performed the display (Fig. 2B). The seasonal differences were clear: more individuals tried to jump away after performing deimatic display in the early season than late season (18% vs. 2%). The tendency to engage in death feigning showed the opposite



Figure 2. Frequency of responses of *Lycorma delicatula* to pecking (A) and grabbing (B) stimulation in two different seasons in a year (and for the two sexes). The early season represents the early adulthood of *L. delicatula* when almost all nymphs have recently become adults, the late season represents the late adulthood of *L. delicatula* near the end of their life-cycle. DD + CW indicates when the insect displayed deimatic display and stayed until it closed its wings. DD + JA indicates the situation when it performed deimatic display, then jumped away subsequently. DF + CW indicates when they performed deimatic display in conjunction with death feigning until it closed its wings (no individual death feigned then jumped away).

trend: only a small proportion of individuals feigned death in the early season, whereas approximately half of the individuals feigned death in the late season (4% vs. 45%). These results indicate that, once they were physically contacted by pecking, their primary choice of post-attack defence was escaping away. However, if the escaping attempt was interfered with through grabbing, then individuals employed a deimatic display or death feigning as a secondary post-attack defence, with the tendency to engage in each of these behaviours (as well as how they ended) varying with sex and season.

Deimatic display duration varied from 1 to 192 s (mean \pm SEM: 15.16 \pm 1.71 s). We found a significant within-individual consistency in deimatic display duration (ICC = 0.77, $F_{14,14}$ = 6.58, P < 0.001).

Reaction latency also varied from 1 to 84 ms (mean \pm SEM: 25.30 \pm 1.79 ms after excluding one outlier with a latency of 284 ms), although we found no evidence of within-individual consistency in reaction latency (ICC = 0.01, $F_{20,20} = 1.01$, P = 0.49). This suggests that, although deimatic display duration was an individual trait, we could not reject the null hypothesis that the reaction latency was not. On the basis of this result, no further investigation of the relationship between reaction speed and tendency to engage in escaping vs. fleeing was conducted.

The results of path analysis of responses to grabbing were broadly consistent with our frequency analysis and are shown in Fig. 3. Only the significant relationships with P < 0.05 are depicted with arrows. The width of each arrow is proportional to the strength of the standardized coefficients. Both sex and season affected the weight of individuals (females were heavier than males: Z = 11.59, P < 0.001; weights of individuals were heavier in the late season than early season, most likely as a result



Figure 3. A, results of path analysis of the responses of *Lycorma delicatula* to grabbing. Only the significant relationships (P < 0.05) are depicted with arrows. Arrow width is proportional to the standardized coefficients; the dashed arrow indicates a marginally significant relationship. Numbers next to arrows are standardized coefficients. The null model description is provided in the Statistical analysis in the main text. B, proportion of the jumped away individuals during deimatic display in the early and the late seasons. C, comparison of the duration of deimatic display between those that did not feign death and those that feigned death in the grabbing experiment.

of the consumption of food: Z = 14.38, P < 0.001). Season also affected both the escape decision of individuals during deimatic display and death feigning behaviour; individuals were more likely to jump away during deimatic display in the early season than the late season (Z = 2.09, P = 0.04) (Fig. 3B), whereas death feigning was more frequently observed in the late season than the early season (Z = 4.89, P < 0.001) (Fig. 3B). The duration of deimatic display was only affected by whether individuals feigned death or not. The duration of the display was significantly longer when the insects additionally feigned death (Z = 8.79, P < 0.001) (Fig. 3C).

As might be expected, signs of wing damage were more frequent in the late season than the early season (GLZ: 86% vs. 10%, Z = 7.79, P < 0.001) and males were more likely to have wing damage than females (GLZ: 54% vs. 40%, Z = 2.99, P = 0.003).

DISCUSSION

The results of the present study clearly show that behavioural anti-predator adaptations of L. delicatula are largely focused on post-attack (i.e. after a physical contact) defences and operate sequentially. Based on our results, the anti-predator adaptations of L. delicatula at each stage of the predation sequence can be summarized (Fig. 4). Lycorma deli*catula* clearly relies on cryptic appearance as a primary defence to avoid detection. Once detected, however, no active defence is performed until it is physically contacted. Recent experiments on artificial prey suggest that post-attack display of distasteful prey can facilitate the predators' learning about the unpalatability of the prey, which subsequently reduces the attack rates of predators on the cryptic normal form of the prey (Kang et al., 2016). Therefore, predators' learned association between cryptic forewings and unpalatable chemicals, facilitated by the deimatic display, may subsequently prevent the predators initiating an attack once it has detected a L. delicatula.

Theoretical arguments suggest that, if the primary defence is highly effective, then there will be no selection for additional defences, a phenomenon known as 'strategy blocking' (Briton *et al.*, 2007). However, it is evident that the pre-attack protection that the bug has evolved via crypsis is not perfect, and our results highlight the fact that *L. delicatula* has evolved multiple post-attack strategies to avoid predation following contact. When initially attacked by a predator, *L. delicatula* primarily tries to escape by rapid jumping away. If jumping away fails (as simulated by grabbing), it uses deimatic display, sometimes in conjunction with death feigning. One



Figure 4. Summary of anti-predator adaptations of *Lycorma delicatula* in different stages of predation sequence. Although not depicted, *L. delicatula* also possess defensive chemicals as an additional anti-predator adaptation, which would make the predators avoid *L. delicatula* upon seeing the cryptic appearance or abandon it upon seeing deimatic display.

advantage of the deimatic display is that it may potentially provide a further 'aide memoire' with respect to the bug's distastefulness (Rothschild, 1984) in that predators are reminded about the prey's defensive chemicals upon seeing the deimatic display. Although evidence for such 'memory jogging' remains equivocal (Ruxton *et al.*, 2004; Kang *et al.*, 2016), it is worth noting that these displays were frequently terminated by individuals closing their wings without any escape attempt.

Lycorma delicatula showed clear seasonal differences in the post-attack defences. There were a number of attempts at jumping away during deimatic display in the early season, whereas jumping away was rarely observed in the late season. By contrast, death feigning terminating in wing closure was more frequently observed in the late season than early season. Accordingly, *L. delicatula* switched their behavioural responses from deimatic-escape to deimatic-warning/feigning strategy as the season progressed. Our results also show that the deimatic display duration was longer on average when individuals feigned death, although this difference was observed mainly because *L. delicatula* maintained deimatic display during death feigning, which consequently increased the duration of deimatic display.

We consider three complementary adaptive explanations for the above behavioural switch in anti-predator responses over the season. First, there might be differences in the foraging mode of local predators between early and late season (Bence, 1986; Nomura et al., 2011). In the early part of the season, when lanternflies have only recently reached adulthood, many predators should be naïve about L. delicatula and its defensive chemicals (or more generally, naïve to aposematic signals; Mappes et al., 2014). Therefore, once detected, L. delicatula may have a high risk of being predated if it does not escape because the associative learning between conspicuous warning display and defensive chemicals has not formed yet in the predators' memory (Mappes et al., 2014). By contrast, in the late season, encountered predators are likely to be educated and so a deimatic display would readily protect L. delicatula without escaping away (Roper & Wistow, 1986).

The second explanation rests on differences in the concentration of defensive alkaloids in adults throughout the season. The host preference of L. delicatula changes from a broad range of plant species during its nymphal stages to a few plant species at the adult stage (Kim et al., 2011). Lycorma delicatula appears to sequester its defensive chemicals from its adult host plants, such as A. altissima (Kim et al., 2011; S. Song, S. I. Lee and P. G. Jablonski, unpubl. data), which contain high concentrations of cvtotoxic alkaloid chemicals (Anderson, Harris & Phillipson, 1983). It is therefore possible that defensive chemicals have not accumulated sufficiently in early-season adults to deter further handling of predators. However, by the time that adults reach the late season, they may have accumulated sufficient defensive chemicals to employ warning signals as an effective deterrent. Chemical analysis on the adult L. delicatula in the early and late season is encouraged to test this hypothesis.

Third, seasonal differences in their responses against grabbing might have appeared because their future reproductive expectations differ between seasons (the 'asset-protection'/finite time horizon principle; Clark, 1994). Thus, in early adulthood, individuals will have a high reproductive expectation, whereas, in late adulthood, if they already participated in their initial reproduction, their future reproductive value is lower. Because individuals with high reproductive expectations tend to be less prone to risk taking than those with low reproductive expectations (Stearns, 1989; Wolf *et al.*, 2007), then individuals might be expected to minimize the risk of predation in the early season (such as by trying jumping away when they had a chance), whereas individuals would show more risk-taking responses in the late season (such as staying after the deimatic display, thereby placing more of its future survival prospects on the choices made by predators).

Both sexes showed similar responses overall, although a small proportion of males did not show any behavioural defences in both seasons, whereas all females showed one (or more) of the active behavioural defences. The presence of these nonresponsive males is puzzling and hard to explain because employing no defence is clearly non-adaptive and the results from wing damage accumulation suggest that males seemed to be attacked (and/or survived the attack) more frequently than females. This nonresponsive behaviour does not appear to be condition dependent because the weights of on-responsive males were similar to those of the other males (GLZ: $\chi_1^2 = 0.62, P = 0.43$). The possibility that individuals could selected to persist post reproduction to educate predators as to their distasteful properties has long been raised (Blest, 1963). However, recent studies suggest that this post-reproductive longevity is unlikely to be maintained by kin selection (Carroll, Korshikov & Sherratt, 2011). Regardless of the reason, these nonresponsive males occupy only a small proportion in a population and we consider that these individuals do not represent population responses.

It would be expected that any prey with postattack defence would have adaptations (e.g. thick cuticles) that enable them to survive the initial attack of predators. The cuticle of L. delicatula appears to be sufficiently durable to endure the probing of an avian predator's attack (C. Kang, unpubl. data; 93/111 lanternflies survived chick's initial attack and handling). This hardiness is important for fully performing the layered defences in L. delicatula because survivorship from the initial predation sequence is required to perform the later defences. Our results also suggest that nonlethal wing damage is accumulated as a season goes on, in that most of the individuals in the late season (86%) had signs of damage on their wings compared to only 10% in the early season. Considering that avian predatory attacks only partly account for the wing damage in winged insects (Shapiro, 1974; Wourms & Wasserman, 1985), we speculate that, during its adult life, each individual would have a high chance of being attacked by predators (which means their primary defence, crypsis, has failed). The evolution of multiple post-attack defences in L. delicatula might then have been driven by the strong pressure for the post-attack defence as a result of the high probability of failure of its crypsis (Bateman et al., 2014). These multiple layers of anti-predator defences might well help explain how this pest insect could invade and establish in new locations.

In conclusion, we have shown that L. delicatula performs its anti-predator adaptations in a stepwise fashion to avoid their final consumption by predators. This stepwise performance of anti-predator behaviours might well have evolved in L. delicatula because each line of defence is imperfect, and each line of defence does not significantly hinder the effectiveness of the pervious lines of defences (e.g. deimatic display does not hinder crypsis, nor escaping behaviour). Of the secondary defences considered, there has been a growing interest in deimatic display (Skelhorn, Holmes & Rowe, 2016; Umbers et al., 2015). Deimatic display in defended species is of special interest because this strategy theoretically can combine the best of both camouflage and aposematism. We anticipate that the protective mechanisms of deimatic display in nondefended prey, such as Catocala moths (Sargent, 1973), and those in defended prey, such as L. delicatula and Acripeza reticulate (Kang et al., 2011; Umbers & Mappes, 2015), should be fundamentally different because the former is a deceiving signal, whereas the latter is an honest signal of unpalatability (Skelhorn et al., 2016). More generally, given that multiple defences (or multiple lines of defences) are generally favoured over a single defence, then we encourage future studies on anti-predator defences to consider the evolution of defensive portfolios as a whole.

ACKNOWLEDGEMENTS

We appreciate H. Lee and J. Moon for their invaluable help during field studies. We thank Tim Caro and an anonymous reviewer for their helpful comments. This study was supported by National Research Foundation of Korea (NRF-2010-0025546, NRF-2013R1A2A2A01006394) and BK-21 program awarded to the School of Biological 415 Sciences, Seoul National University. CK is supported by Natural Sciences and Engineering Research Council of Canada Discovery Accelerator Supplement Program (NSERC DAS).

REFERENCES

- Anderson LA, Harris A, Phillipson JD. 1983. Production of cytotoxic canthin-6-one alkaloids by Ailanthus altissima plant cell cultures. Journal of Natural Products 46: 374–378.
- Animal Behavior Society. 2012. Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour* 83: 301–309.
- Bateman AW, Vos M, Anholt BR. 2014. When to defend: antipredator defenses and the predation sequence. *Ameri*can Naturalist 183: 847–855.

- Beck CA, Iverson SJ, Bowen W, Blanchard W. 2007. Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. *Journal of Animal Ecology* **76**: 490–502.
- **Bence JR. 1986.** Feeding rate and attack specialization: the roles of predator experience and energetic tradeoffs. *Environmental Biology of Fishes* **16:** 113–121.
- Bilde T, Tuni C, Elsayed R, Pekár S, Toft S. 2006. Death feigning in the face of sexual cannibalism. *Biology Letters* 2: 23–25.
- **Blest AD. 1963.** Longevity, palatability and natural selection in five species of new world Saturniid moth. *Nature* **197:** 1183–1186.
- Briton NF, Planqué R, Franks NR. 2007. Evolution of defence portfolios in exploiter-victim systems. Bulletin of Mathematical Biology 69: 957–988.
- **Broom M, Ruxton GD. 2005.** You can run or you can hide: optimal strategies for cryptic prey against pursuit predators. *Behavioral Ecology* **16:** 534–540.
- Brown SG, Boettner GH, Yack JE. 2007. Clicking caterpillars: acoustic aposematism in *Antheraea polyphemus* and other Bombycoidea. *Journal of Experimental Biology* 210: 993–1005.
- Buitron D. 1983. Variability in the responses of black-billed magpies to natural predators. *Behaviour* 87: 209–235.
- **Caro T. 2005.** Antipredator defenses in birds and mammals. Chicago, IL: University of Chicago Press.
- Carroll J, Korshikov E, Sherratt TN. 2011. Post-reproductive senesence in moths as a consequence of kin selection: Blest's theory revisted. *Biological Journal of the Linnean Society* 104: 633–641.
- Clark CW. 1994. Antipredator behavior and the asset-protection principle. *Behavioral Ecology* 5: 159–170.
- **Cott HB. 1940.** Adaptive coloration in animals. London: Methuen.
- Dara SK, Barringer L, Arthurs SP. 2015. Lycorma delicatula Hemiptera: Fulgoridae: a new invasive pest in the United States. Journal of Integrated Pest Management 6: 20.
- Edmunds M. 1974. Defence in animals: a survey of anti-predator defences. New York, NY: Longman Publishing Group.
- Endler JA. 1991. Interactions between predators and prey. In: Krebs JR, Davis NB 3rd, eds. *Behavioural ecology an* evolutionary approach. Oxford: Blackwell Scientific Publications, 169–196.
- Han JM, Kim H, Lim EJ, Lee S, Kwon Y-J, Cho S. 2008. Lycorma delicatula Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae finally but suddenly arrived in Korea. Entomological Research 38: 281–286.
- Hileman KS, Brodie ED. 1994. Survival strategies of the salamander *Desmognathus ochrophaeus*: interaction of predator-avoidance and anti-predator mechanisms. *Animal Behaviour* 47: 1–6.
- Honma A, Oku S, Nishida T. 2006. Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. *Proceedings of the Royal Society of London Series B, Biological Sciences* 273: 1631– 1636.

- Kang C-K, Lee S-I, Jablonski PG. 2011. Effect of sex and bright coloration on survival and predator-induced wing damage in an aposematic lantern fly with startle display. *Ecological Entomology* **36**: 709–716.
- Kang C, Cho H-J, Lee S-I, Jablonski PG. 2016. Postattack aposematic display in prey facilitates predator avoidance learning. *Frontiers in Ecology and Evolution* 4: 35.
- Kim JG, Lee E-H, Seo Y-M, Kim N-Y. 2011. Cyclic behavior of Lycorma delicatula insecta: Hemiptera: Fulgoridae on host plants. Journal of Insect Behavior 24: 423–435.
- Kim H, Kim M, Kwon DH, Park S, Lee Y, Huang J, Kai S, Lee H-S, Hong K-J, Jang Y, Lee S. 2013. Molecular comparison of Lycorma delicatula (Hemiptera: Fulgridae) isolates in Korea, China, and Japan. Journal of Asia-Pacific Entomology 16: 503–506.
- Lenzi-Mattos R, Antoniazzi MM, Haddad CFB, Tambourgi DV, Rodrigues MT, Jared C. 2005. The inguinal macroglands of the frog *Physalaemus nattereri* Leptodactylidae: structure toxic secretion and relationship with deimatic behaviour. *Journal of Zoology* **266**: 385–394.
- Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68: 619–640.
- Loehlin JC. 2011. Latent variable models: an introduction to factor path and structural analysis, 4th edn. New York, NY: Lawrence Erlbaum Associates Inc.
- Mappes J, Kokko H, Ojala K, Lindström L. 2014. Seasonal changes in predator community switch the direction of selection for prey defences. *Nature Communications* 5: 5016.
- Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M. 2004. Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. Proceedings of the Royal Society of London Series B, Biological Sciences 271: 2293–2296.
- Nomura F, do Prado VHM, da Silva FR, Borges RE, Dias NYN, de Rossa-Feres DC. 2011. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. *Journal of Zoology* **284**: 144–150.
- Ohno T, Miyatake T. 2007. Drop or fly? Negative genetic correlation between death-feigning intensity and flying ability as alternative anti-predator strategies. *Proceedings of the Royal Society of London Series B, Biological Sciences* 274: 555–560.
- **Olofsson M, Eriksson S, Jakobsson S, Wiklund C. 2012.** Deimatic display in the European swallowtail butterfly as a secondary defence against attacks from great tits. *PLoS ONE* **7**: e47092.
- **R Core Team. 2014.** *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. Available at: http://www.r-project.org.
- Rogers SM, Simpson SJ. 2014. Thanatosis. Current Biology 24: R1031–R1033.
- Romanes GJ, Darwin C. 1883. Mental evolution in animals. New York, NY: D. Appleton and Company.
- Roper TJ, Wistow R. 1986. Aposematic colouration and avoidance learning in chicks. *Quarterly Journal of Experi*mental Psychology 38: 141–149.

- **Rosseel Y. 2012.** lavaan: An R package for structural equation modeling. *Journal of Statistical Software* **48**: 1–36.
- Rothschild M. 1984. Aide memoire mimicry. *Ecological Entomology* 9: 311–319.
- Ruxton GD, Sherratt TN, Speed MP. 2004. Avoiding attack. Oxford: Oxford University Press.
- Sargent TD. 1973. Studies on the Catocala Noctuidae of southern New England. IV. A preliminary analysis of beakdamaged specimens with discussion of anomaly as a potential anti-predator function of hindwing diversity. Journal of the Lepidopterists' Society of the United States of America 27: 175–192.
- Schlenoff DH. 1985. The startle responses of blue jays to Catocala Lepidoptera: Noctuidae prey models. Animal Behaviour 33: 1057–1067.
- Shapiro AM. 1974. Beak-mark frequency as an index of seasonal predation intensity on common butterflies. American Naturalist 108: 229–232.
- Silveira HCP, Oliveira PS, Trigo JR. 2010. Attracting predators without falling prey: chemical camouflage protected honeydew-producing treehoppers from ant predation. *American Naturalist* 175: 261–268.
- Skelhorn J, Holmes GG, Rowe C. 2016. Deimatic or aposematic? Animal Behaviour 113: e1-e3.
- Stearns SC. 1989. Trade-offs in life-history evolution. *Functional Ecology* 3: 259–268.
- Thompson RKR, Foltin RW, Boylan RJ, Sweet A, Graves CA, Lowitz CE. 1981. Tonic immobility in

Japanese quail can reduce the probability of sustained attack by cats. *Animal Learning Behavior* **9:** 145–149.

- Umbers KDL, Mappes J. 2015. Postattack deimatic display in the mountain katydid Acripeza reticulata. Animal Behaviour 100: 68–73.
- Umbers KDL, Lehtonen J, Mappes J. 2015. Deimatic displays. Current Biology 25: R58–R59.
- Vallin A, Jakobsson S, Lind J, Wiklund C. 2005. Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. *Proceedings of the Royal Society of London Series B, Biological Sciences* 272: 1203–1207.
- Wilson AP, Boelkins RC. 1970. Evidence for seasonal variation in aggressive behaviour by Macaca mulatta. Animal Behaviour 18: 719–724.
- Wolf M, van Doorn GS, Leimar O, Weissing FJ. 2007. Life-history trade-offs favour the evolution of animal personalities. *Nature* 447: 581–584.
- Wourms MK, Wasserman FE. 1985. Bird predation on Lepidoptera and the reliability of beak-marks in determining predation pressure. Journal of the Lepidopterists' Society of the United States of America **39**: 239–261.
- Xue G, Yuan S. 1996. Separation and preparation of indole alkaloids in *Lycorma delicatula* White. by HPLC. *China Journal of Chinese Materia Medica* 21: 554–555.
- Ydenberg RC, Dill LM. 1986. The economics of fleeing from predators. Advances in the Study of Behavior 16: 229– 249.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. The proportion of individuals that jumped away in response to the various nontactile stimuli.
Figure S2. Null model for the path analysis of the responses of *Lycorma delicatula* to grabbing.
Video S1. A movie that shows deimatic display response of *L. delicatula* in response to a tactile stimulus.
Video S2. A movie that shows jumping away response of *L. delicatula* in response to a tactile stimulus.
Video S3. A movie that shows death feigning response of *L. delicatula* in response to a tactile stimulus.