LETTER

Selective flower abortion maintains moth cooperation in a newly discovered pollination mutualism

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Abstract

The evolutionary stability of mutualisms is enhanced when partners possess mechanisms to prevent overexploitation by one another. In obligate pollination–seed consumption mutualisms, selective abortion of flowers containing excessive eggs represents one such mechanism, but empirical tests have long been limited to the yucca–yucca moth mutualism. We present evidence for selective abortion in the recently discovered mutualism between *Glochidion* trees and *Epicephala* moths. In *Glochidion acuminatum*, proportion of aborted flowers progressively increased both with higher egg load and increased ovule damage. Selective abortion resulted in a 16% seed production increase compared with expectations under random abortion, and moths suffered fitness losses as high as 62% when ovipositing into pre-infested flowers. Moth eggs were laid singly more often than expected under random oviposition, thus avoiding potential disadvantages from multiple infestations. As new pollination mutualisms are being discovered, selective abortion mechanisms may prove to be more widespread than previously thought.

INTRODUCTION

Explaining the evolutionary stability of interspecific mutualisms remains one of the major challenges in evolutionary biology (Sachs *et al.* 2004; West *et al.* 2007; Kiers & Denison 2008). Mutualisms are best viewed as balanced antagonistic interactions that nonetheless provide net benefits to both species. When several partners interact with a host, selection should thus favour less-mutualistic partners that obtain more benefits while paying fewer costs, leading to a potential collapse of the cost-benefit balance. Theoretical models predict that the evolutionary stability of mutualisms is greatly enhanced when participants employ mechanisms to prevent excessive exploitation by the other (Axelrod & Hamilton 1981; Bull & Rice 1991; West *et al.* 2002a,b). However, empirical demonstration of such mechanisms has proven difficult and is still limited to a few species (Pellmyr & Huth 1994; Kiers *et al.* 2003; Bshary & Grutter 2006).

The mutualistic association between yuccas and yucca moths is one of the most iconic systems in the study of interspecific cooperation. Yuccas are exclusively pollinated by yucca moths, who in exchange for oviposition within flowers, actively deliver pollination services; the hatching larvae then consume a subset of the resulting seeds (Pellmyr 2003). When a yucca moth lays too many eggs on a single flower, a high proportion of seeds will be eaten by pollinator larvae, hampering plant reproduction and potentially destabilizing the mutualism. Some yucca species selectively abort flowers with high egg loads, thereby allocating resources to flowers with fewer ovipositions (Pellmyr & Huth 1994; Addicott & Bao 1999; Shapiro & Addicott 2004). Although selective abortion is a plant mechanism to increase its own fitness, it is also expected to facilitate the long-term stability of the mutualism in two ways; non-random abortion prevents moths from laying heavy egg loads on flowers (Huth & Pellmyr 1999), while at the same time, it regulates moth population sizes and thereby reduces future probability of being overexploited (Holland & DeAngelis 2002, 2006).

Selective flower abortion does not, however, provide a general explanation for the stability of all pollination–seed

consumption mutualisms. Selective abortion has only recently been documented in the fig–fig wasp mutualism (Herre *et al.* 2008; Tarachai *et al.* 2008) and the less-obligate *Silene–Hadena* mutualism (Jolivet & Bernasconi 2006; Burkhardt *et al.* 2009). It is virtually non-existent in other systems, including the senita–senita moth (Holland *et al.* 2004a) and globeflower–globeflower fly (Jaeger *et al.* 2000) interactions. Even within the yucca–yucca moth mutualism, abortion mechanisms do not occur in yucca flowers infested by shallow-ovipositing moth species (Wilson & Addicott 1998; Addicott & Bao 1999). Consequently, research interests have shifted away from selective abortion hypotheses and moved to extrinsic elements and third-party interactions as potential factors stabilizing these mutualistic associations (Segraves 2003, 2008; Althoff *et al.* 2005; Crabb & Pellmyr 2006). However, the relative importance of selective abortion in stabilizing pollination–seed consumption mutualisms requires further assessment, especially given that novel obligate pollination mutualisms are still being discovered today (Kato *et al.* 2003; Kawakita & Kato 2004a,b, 2009). Insights from these other systems have the potential to enhance our understanding of how and to what extent selective abortion contributes to long-term persistence of mutualisms.

In this study, we present evidence for the first known case of host-mediated selective abortion in the newly discovered pollination mutualism between Phyllanthaceae plants and *Epicephala* moths (Gracillariidae; Kato *et al.* 2003). We focused on the interaction between *Glochidion acuminatum* Mull. Arg. and its obligate seed-parasitic *Epicephala* moth pollinator. In this system, similar to yucca moths, females of the species-specific pollinator, *Epicephala* sp., actively collect and transport pollen with specialized proboscis and insert an egg into the pistil after pollination. The larvae hatch in the developing flowers, each consuming a subset of the six seeds within a fruit to complete larval development (Kato *et al.* 2003).

This association shares several features with the yucca system that make it a likely candidate for selective abortion to occur, including (i) deep oviposition into floral tissue, which may act as a cue to detect moth oviposition (Addicott & Bao 1999; Marr & Pellmyr 2003), and (ii) variation in the number of larvae coexisting within a single ovary, a requirement for any abortion to be selective. Our results are discussed in light of the overall effects of selective abortion on retention probability of pollinated flowers, seed production and survivorship of *Epicephala* eggs.

MATERIALS AND METHODS

Study site and species

The study was conducted at Nagakumo-toge (28°26′ N, 129°35′ E), Tatsugo, Amami-Ohshima Island, Japan, in 2007 and 2008. *Glochidion acuminatum* is a monoecious tree

that occurs in forest margins of tropical and subtropical forests in eastern Asia (Govaerts *et al.* 2000). The plants attain a height of 3–8 m and produce tens to hundreds of thousands of male and female flowers from early May to the middle June. Flowering is synchronous both between sexes and among individuals. Male flowers are pedunculate with unfolded yellow perianths and connate ellipsoid stamens, whereas female flowers are inconspicuously green, sessile and composed of reduced perianths and fused styles. The styles form a narrow pit at the tip of the fused column, into which *Epicephala* females deposit pollen and insert the ovipositor. The length of female flower and diameter of the stylar column are 2.49 ± 0.08 mm (mean $\pm$ standard error) and 1.09 ± 0.02 mm respectively ($n = 12$, one flower measured per plant). Flowers are produced in axillary clusters on each branch with a tendency of female flowers to occur towards the apex. Each cluster typically consists of 10–20 flowers. Single flowers last *c.* 1 week. The majority of flower abscission (including abortion) occurs by late June, soon after anthesis. Pollinated flowers undergo *c.* 3 months of dormancy and begin to develop in late September, and fruits become mature by late November. Each flower contains six ovules (Kato *et al.* 2003).

The moth, *Epicephala* sp., is the only known pollinator of *G. acuminatum* (Kato *et al.* 2003; Kawakita & Kato 2006). Male and female moths do not differ in size and have forewing lengths of 4.85 ± 0.04 mm ($n = 6$) and 4.76 ± 0.05 mm ($n = 6$) respectively. At night, an *Epicephala* female moth visits male flowers to collect pollen by rubbing the proboscis against the anthers. Upon visiting female flowers, a female uncoils its proboscis and repeatedly pushes the proboscis tip into the stylar pit to pollinate the flowers; in doing so, it recoils the proboscis several times to replenish pollen on the proboscis tip. After pollination, the female moth bends its abdomen to insert the ovipositor through the apical pit into the stylar tissue. An *Epicephala* female visits *c.* 20 female flowers sequentially in a single oviposition bout, repeating the above stereotypic behaviour on each female flower. *Epicephala* eggs remain dormant from June to late September, after which they hatch, and larvae develop, as flowers start developing into fruits. Mature larvae emerge from fruits, pupate and overwinter in the litter, and emerge as adults in next May. *Epicephala* larvae have not been observed to exit and reenter fruits, even when a larva faces seed limitation within a single fruit.

Pollination experiment

For selective abortion to occur, plants must abscise a fraction of their pollinated flowers as a result of resource limitations occurring after pollination events. To investigate if *G. acuminatum* plants abscise flowers and that abscission is not solely due to pollination limitations, we ran pollen

supplementation experiments. We supplemented outcross pollen on 26–62 female flowers on each of seven reproductive individuals in May 2008 and compared their survivorship with 26–67 female control flowers per plant. Flowers were monitored bi-weekly until late June and monthly thereafter until fruit maturation in November. To determine natural pollination and abortion rates, we also estimated the proportion of flowers pollinated at the time of peak flowering and compared this with naturally occurring fruit set, which is the survivorship of control flowers as described earlier. Proportion of flowers pollinated was determined by checking for pollen grains on stigmas under a dissecting microscope in 30 flowers randomly sampled from each of seven plants.

Test of selective abortion

To test for the occurrence of selective abortion, we randomly sampled 50 retained and 50 aborted flowers from each of five *G. acuminatum* plants in 2007. To collect aborted flowers, we set up basket traps (1 mm mesh; entrance, 600 mm × 900 mm; depth, 300 mm) under the trees during the main period of flower abortion (from 15 May to 10 June). We distinguished pollinated and unpollinated flowers by staining pollen attached to the stigma using aniline blue (Dafni *et al.* 2005). We collected female flowers retained on branches in late August when abortion ends and flowers begin to develop into fruits. We dissected aborted and retained flowers under a microscope, counting both the numbers of eggs and oviposition scars per flower. There was a near 1:1 correspondence between numbers of scars and eggs (2 of 574 scars without eggs). Thus, egg number was deemed a reliable indicator of oviposition by *Epicephala*.

When inserting eggs, female *Epicephala* moths can sometimes pierce too deeply and penetrate the ovule. Therefore, we also counted the number of ovules damaged by penetration of moth eggs. To test whether the numbers of eggs and damaged ovules per flower differ significantly between aborted and retained flowers, we used generalized linear mixed models (GLMMs), which allow both fixed and random factors to be fitted to the model, with random factors taking into account repeated measures within plant. We incorporated the number of eggs/damaged ovules as the dependent variable, floral status (aborted/retained) as a fixed factor and plant as a random factor into the model. GLMMs were conducted with the glmmML function of R (ver. 2.9.2; R Development Core Team 2009).

Assessment of the effects of selective abortion

Based on the frequency distribution of eggs in retained and aborted flowers, we investigated how selective abortion affects overall fitness of plants and moths. Because fitness to an individual (either plant or moth) is assessed by the number of offspring that survive to the next generation, we estimated the increase/decrease in each of the following fitness parameters as compared to when abortion was random: (i) overall seed production by plants, (ii) population-level survivorship of moth eggs, and (iii) retention probabilities of flowers with one egg and those with more than one egg. Because we sampled equal numbers of retained and aborted flowers, we adjusted for natural occurrence of the proportion of retained/aborted pollinated flowers. Due to difficulties in distinguishing pollinated flowers in the field and monitoring them throughout the reproductive season, this parameter was estimated based on the proportion of female flowers that were pollinated at peak anthesis (May) and retention probability of naturally pollinated flowers (November), as described earlier. To assess the overall increase in seed production, we estimated the expected proportions of seeds that will remain intact in flowers infected with different numbers of eggs. This was performed by enumerating the numbers of uneaten and predated seeds per fruit in a total of 100 fully matured fruits in November 2008, sampled from five plants that had received variable numbers of moth ovipositions. Number of ovipositions can be accurately counted in fully matured fruits by dissecting the stylar column and checking for oviposition scars.

Detailed procedures for estimating the effects of selective abortion on plant and moth fitness are provided in Appendix S1.

Analysis of moth egg distribution among flowers

If *G. acuminatum* selectively aborts flowers with excessive eggs, *Epicephala* moths would benefit from avoiding laying eggs in pre-infested flowers. To test if *Epicephala* females avoid ovipositing into flowers containing eggs, we compared the frequency distribution of flowers with different numbers of eggs to that expected under random oviposition (Poisson distribution) using a chi-squared test. A total of 210 flowers collected from seven plants in May 2008 were used for this analysis. Because a chi-square test does not distinguish whether the observed egg distribution is uniform or clumped, an additional test using Morishita's aggregation index (Morishita 1959) was performed. Morishita's index, $I\delta$, is defined as

$$I\delta = Q \sum_{i=1}^{Q} \frac{x_i(x_i - 1)}{N(N - 1)},$$

where Q is the total number of flowers, x_i is the number of eggs in the *i*th flower and N is the total number of eggs. The observed $I\delta$ is tested against the expected value under a Poisson distribution using an *F*-test (Morishita 1959). Eggs

are uniformly distributed among flowers if the observed $I\delta$ is significantly < 1.

RESULTS

Glochidion acuminatum flower abscission was found not to be due to pollination limitations. At time of peak flowering in May, $75.7 \pm 6.7\%$ (mean $\pm$ standard error, $n = 7$) of all female flowers were pollinated naturally. Despite ample pollination, plants aborted a high percentage of pollinated flowers, with low numbers of flowers retained in both artificially outcrossed ($25.5 \pm 5.1\%$, $n = 7$) and control flowers ($7.6 \pm 4.0\%$, $n = 7$) by late June (Fig. 1). At this same time point, proportion of retained flowers was higher in artificially outcrossed than control flowers (Mann–Whitney U-test, $P < 0.01$), but this result is thought to arise from the retention of higher quality flowers in the outcrossed treatment (see Discussion). Proportions of retained flowers remained largely unchanged from late June to November (Fig. 1). Flowers begin to develop, and *Epicephala* larvae hatch, in late September (Fig. 1).

Floral abortion in *G. acuminatum* was found to be highly non-random. Aborted flowers contained greater than random proportions of two-, three- and four-egg flowers (two: 68.0%, $n = 75$; three: 85.7%, $n = 14$; four: 100%, $n = 1$), whereas retained flowers contained greater than random proportion of zero-egg flowers (79.4%, $n = 34$) (Fig. 2). Similarly, damaged ovules were not randomly distributed, with 85.0% ($n = 80$) and 100% ($n = 14$) of the flowers with one and two damaged ovules, respectively, from aborted flowers (Fig. 2). GLMMs indicate significant effects of floral fate (aborted/retained) on number of eggs (Wald test: $z_1 = -2.92$, $P < 0.01$) and number of damaged ovules ($z_2 = -6.68$, $P < 0.001$) per flower.

To determine the fitness benefits of selective abortion for host plants, we compared the expected proportion of uneaten seeds per fruit vs. estimations under random abortion. Estimations were made using data from the relative frequencies of eggs in retained and aborted flowers
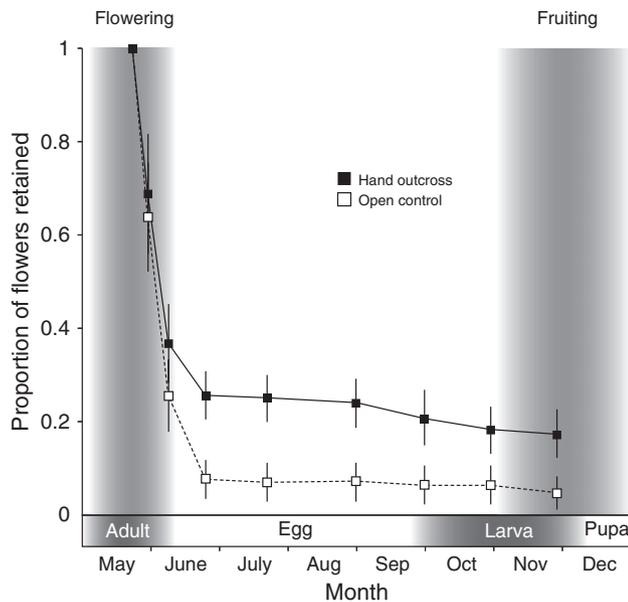
(a)

(b)

Figure 2 Selective flower abortion based on number of eggs and number of damaged ovules. We sampled 50 aborted and 50 retained flowers from each of five plants and sorted the flowers by (a) the number of eggs carried and (b) the number of ovules penetrated by moth ovipositor. Data plot shows the relative proportion of retained flowers within the sample for each number class. If abortion is random, the regression line should approximate to $y = 0.5$ (broken line). Solid lines indicate logistic regressions of flower fate (0, aborted; 1, retained) on (a) eggs per flower ($y = 1/(1 + e^{-(-1.104+0.976x)})$) and (b) damaged ovules per flower ($y = 1/(1 + e^{-(-0.336+2.083x)})$). Size of the data plots is proportional to relative frequency.

Figure 1 Survivorship of *Glochidion acuminatum* flowers from anthesis to fruit maturity. *Glochidion acuminatum* produces tens to hundreds of thousands of male and female flowers from early May to middle June. Female flowers normally last *c.* 1 week. *Epicephala* moth eggs remain dormant during the summer until pollinated flowers begin to develop in late September. Filled and open boxes indicate changes in the proportion of artificially cross-pollinated and control female flowers that are retained on branches respectively ($n = 7$). Corresponding life stages of *Epicephala* moth are provided in the bottom bar.
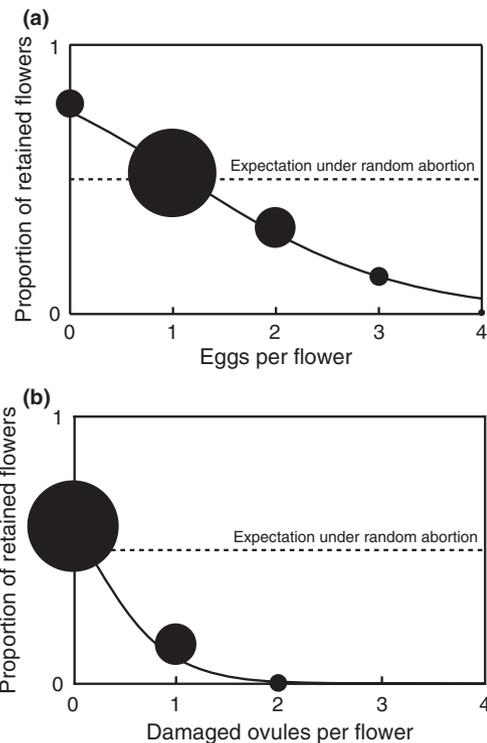
(Table S1), the average proportion of pollinated female flowers that were retained to fruiting stage (0.054 ± 0.043, $n = 7$), and the average proportion of uneaten seeds in fruits with one egg (61.4 ± 2.99%, $n = 77$) and two eggs (22.9 ± 6.98%, $n = 17$) (Table S2). Because fruits hosting three or more eggs were extremely rare in natural crops, we conservatively used the proportion of seeds survived two larvae as alternative for the proportions of seeds survived three or more larvae. Based on these data, we found that under random abortion, 53.1% of seeds per fruit would be uneaten, whereas we observed 61.6% intact seeds per fruit. This represents a 15.9% increase in seed production (Table 1). Although the proportion of pollinated female flowers retained to fruiting stage varies greatly among plants in our study population (0–37.0%), the estimated fitness advantage remains largely constant across a range of fruit set (11.2–17.0%; Fig. 3a).

A comparable degree of negative effects on population-level survivorship of moth eggs due to non-random abortion were also noted. On average, 1.27 eggs were laid per pollinated flower. Of these, 5.4% would survive to the fruiting stage if abortion was random. In actuality, we found that only 4.3% survived, suggesting that selective abortion decreases moth survivorship by 20.9%, a value largely unaffected by variation in fruit set (16.2–21.8%; Fig. 3b).

Additionally, based on the average proportion of pollinated female flowers that were retained to fruiting stage (0.054 ± 0.043, $n = 7$) and the relative frequencies of eggs in retained and aborted flowers (Table S1), we estimate that, on average, *Epicephala* females have retained 5.9% of their offspring to fruiting stage when ovipositing in virgin flowers vs. 2.3% when moths oviposit in flowers already containing egg(s). This represents a 61.7% decrease in offspring survival for moths ovipositing in pre-infested flowers (Table 1). Considering that natural fruit set varies among plants between 0% and 30.7% of total flowers pollinated, the estimated increase in the proportion of lost-offspring due to multiple egg laying ranges between 53.5% and 63.1% (Fig. 3c).

Lastly, we found that *Epicephala* females lay eggs evenly among flowers, with 126 of the 210 flowers examined having only one egg. The observed egg distribution was

Table 1 Outcomes of costs and benefits resulting from selective abortion (%)

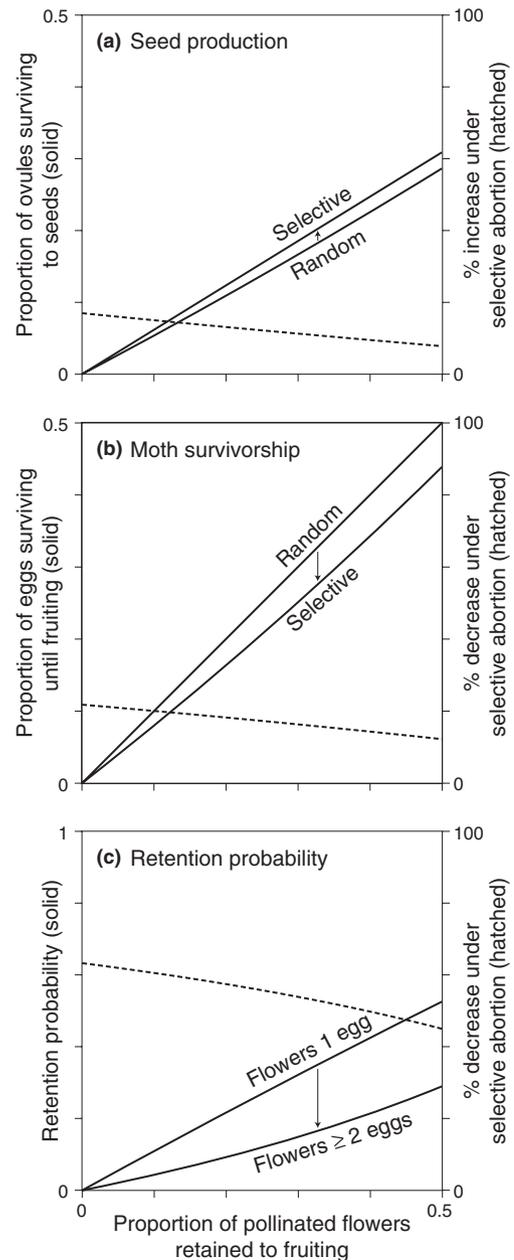|	Random abortion	Selective abortion
Proportion of uneaten seeds per fruit	53.1	61.6
Population-level moth survivorship	5.4	4.3
Retention probability of flowers with 1 egg	5.4	5.9
Retention probability of flowers with > 1 egg	5.4	2.3

Figure 3 Effects of selective flower abortion on plant and moth fitness. (a) Solid lines indicate estimated proportions of total ovules formed that survive to seeds per plant under random and selective abortion. Hatched line represents percentage increase in seed production under selective abortion relative to random abortion. (b) Solid lines are estimated proportions of total moth eggs laid that survive until fruiting season under random and selective abortion. Hatched line represents percentage decrease in population-level moth survivorship under selective abortion. (c) Solid lines indicate estimated retention probabilities of flowers with one egg and those with more than one egg under selective abortion. Percentage decrease in the probability of retention is depicted by the hatched line. Full details of the procedures used to obtain the curves are provided in Appendix S1.

significantly different from a Poisson distribution ($\chi^2 = 51.2$, $P < 0.01$) and more uniform than expected under random oviposition (Morishita's index, $I\delta = 0.44$, $F = 2.00$, $P < 0.01$). These data suggest that moths prefer to lay eggs in virgin flowers and avoid those pre-occupied, in accords with the substantial disadvantage of losing fruits containing multiple eggs.

DISCUSSION

One prerequisite for effective selective abortion is that plants abscise a fraction of their pollinated flowers as a result of resource limitations occurring after pollination. Evidence that *G. acuminatum* plants were not pollen-limited is given by the fact that natural pollination occurred in 76% of all female flowers at the time of peak flowering in May. However, by late June, the proportion of these naturally pollinated flowers retained on branches dropped below 8% (Fig. 1). Therefore, in total, despite receiving sufficient pollination services, *G. acuminatum* routinely drop *c.* 90% of all pollinated flowers within the short period following anthesis. The observed higher fruit set in artificially outcrossed flowers compared to those pollinated naturally can in theory, be interpreted, as an indication of pollen limitation. However, because artificially pollinated flowers received abundant pure outcross pollen, and because a fraction of these flowers are free of *Epicephala* eggs, it is likely that plants allocated more resources to these 'high-quality' flowers, resulting in a comparatively higher fruit set. Pollen-mediated differential maturation occurs under limited resources (Huth & Pellmyr 2000) and often confounds inference of resource limitation (Zimmerman & Pyke 1988; Ashman *et al.* 2004; Knight *et al.* 2005). The most rigorous test of pollen-limitation would be to manipulate all the flowers on single plants and compare fruit sets with those of unmanipulated plants – an approach that is impossible in our study system given that *Glochidion* produces thousands of female flowers in a very short period. In any case, the observed high percentage of pollinated, yet still abscised flowers provides ample opportunity for the plants to selectively mature flowers based on resource limits arising from differential costs of the mutualism.

A second prerequisite of a selective abortion mechanism is that the abortion is indeed 'selective', and based on cost (e.g. egg load or ovule damage), rather than random or based on factors unrelated to the mutualism (e.g. pollen quantity/quality). Our data suggest that the proportion of aborted flowers increases progressively both with higher egg load and with increased ovule damage (Fig. 2). Because flowers with more eggs receive greater amount of pollen of potentially higher paternal diversity, differential pollination quantity/quality unlikely explains the higher abortion rates in flowers with more eggs. These results provide the first evidence that *G. acuminatum* trees selectively abort pollinated flowers based on cost inflicted by *Epicephala* moth ovipositions.

A third requirement for selective abortion to be a mechanism preventing overexploitation is that there be an immediate fitness benefit to individual plants employing selective abortion. In fact, we found that with selective abortion, *G. acuminatum* gains a 16% fitness increase in seed production. These results are similar to the pattern previously reported in yuccas (Pellmyr & Huth 1994; Wilson & Addicott 1998; Addicott & Bao 1999; Shapiro & Addicott 2004); retention probability decreases as more eggs per flower are laid, which improves overall seed production and plant fitness.

Lastly, selective abortion will be most effective in stabilizing cooperation when moths that defect from the mutualism incur a relatively higher fitness loss than those that cooperate. What does it mean for a moth to defect from a pollination mutualism? *Glochidion acuminatum* flowers receive sufficient pollen from one moth visit to fertilize all six of their ovules (Kato *et al.* 2003). Therefore, moths that lay eggs in pre-infested, pre-pollinated flowers are essentially defecting from their mutualistic duties because they burden the host with extra larval costs but deliver no measurable pollination benefit.

However, for the moths to benefit, they must also gain an advantage from this defection, perhaps by avoiding costs of pollination. Cost of pollination (i.e., collection, transportation and deposition of pollen using a proboscis) to an individual moth is difficult to estimate (Pellmyr 1997a). There is indirect support for the idea that pollination is costly in both time and energy for moths in related pollination systems; this is thought to have lead to a complete loss of mutualistic traits in one derived clade of *Epicephala* moths (Kawakita & Kato 2009). Similarly, loss of mutualistic behaviour has been documented in yucca moth (Addicott 1996; Pellmyr *et al.* 1996; Pellmyr & Krenn 2002), suggesting that pollination behaviour can be costly.

Our data suggest that female moths can avoid ovipositing into flowers pre-infested with eggs. We found that *Epicephala* eggs are laid more evenly than expected under random oviposition, indicating that moth females prefer virgin flowers and avoid those visited previously. Our data, however, do not necessarily distinguish between whether moths detect previous oviposition *per se* or use indirect cues correlated with presence of eggs (post-pollination changes in floral attractants, floral age, etc.). Regardless, we suggest that *Epicephala* moths are capable of 'actively' defecting from pollination because they can effectively avoid flowers pre-infested by other moths. A critical question, then, is whether the cost inflicted by selective abortion is large enough to swamp the benefit gained by avoiding the cost of pollination. We estimate that under selective abortion mechanisms,

Epicephala moths suffer more than a half-fold decrease in the probability of progeny survival due to multiple egg laying (Fig. 3). Given this fitness loss, selection against moths that defect from pollination seems highly likely.

Yucca moths are suggested to have pheromonal means of detecting floral egg status and thereby laying fewer eggs in pre-infested flowers (Huth & Pellmyr 1999). The ability to avoid conspecific egg load is not, however, unique to obligate pollination mutualists – a large number of lepidopteran taxa in which resource-limited competition reduces larval performance are known to disperse eggs evenly among resource patches (Thompson & Pellmyr 1991; Holland *et al.* 2004c). Thus, the ability to avoid pre-infested flowers in *Epicephala* may not be an adaptation that has evolved in response to selection pressures imposed by selective abortion. Further studies of oviposition behaviour in related *Epicephala* not suffering from selective abortion would help us understand whether avoiding multiple infestations is a ubiquitous trait within *Epicephala*.

Another important question is whether selective abortion is a mechanism employed by *Glochidion* exclusively to control *Epicephala* moths or a more general mechanism to selectively abort flowers based on other types of damage. Although yucca species can selectively abort flowers based on amount of egg load, flowers can also be aborted due to damage caused by other insects in all close relatives of yuccas (Pellmyr 1997b; Bronstein 2003). This means that initially, selective abortion in yuccas might not have evolved solely as a mechanism for pollinator control, but may have been secondarily co-opted in this context. The prevalence of selective abortion in close relatives of *Glochidion* with non-*Epicephala* pollination systems has yet to be determined, but these investigations will add more insight into how selective abortion mechanisms are integrated with other potential factors, to shape the evolutionary stability of cooperation between plants and their seed-parasitic pollinators.

Studies of other obligate pollination–seed consumption mutualisms suggest that selective abortion is not the only mechanism to enhance mutualistic pollinator behaviour. In figs, experimental introduction of pollen-free wasps shows that the wasps often do leave viable progeny in unpollinated figs that are not aborted, yet they still suffer reduced reproductive output, indicating that selection against wasps that do not pollinate can take other forms than selective abortion (Herre *et al.* 2008). However, unpollinated flowers are invariably abscised in *Glochidion* as well as in all other plants pollinated by seed-parasitic pollinators (e.g. yuccas), suggesting that the same mechanism is unlikely in non-fig systems. Importantly though, selective abortion alone may not be the sole mechanism maintaining cooperation in these obligate mutualisms.

One distinct feature of *Glochidion* plants that deserves further attention is that they produce a large excess of female flowers, far above the number that can become fruits (Fig. 1). This appears paradoxical given the fact that *Glochidion* fruit set is strongly limited by resource. However, a high flower-to-fruit ratio is a common feature of yucca, senita cactus, and *Glochidion* systems and may be an indication that excess female flowers act as a sink for moth eggs, a mechanism that has been proposed to also regulate moth populations (Holland & DeAngelis 2002, 2006; Holland *et al.* 2004b). Selective abortion mechanisms further contribute to population regulation by exerting an additional 21% decrease in moth egg survival compared to random abortion (Fig. 3). Holland *et al.* (2004b) used model simulations to show that excess flower production and fruit abortion, either selective or random, can be an evolutionary stable strategy that regulates moth population in hermaphroditic hosts (e.g. yuccas and senita cacti). The evolutionary conditions allowing stable production of excess female flowers in monoecious hosts (e.g. *Glochidion*) requires further study.

Our finding that selective abortion exists in a novel pollination system that is unrelated to yuccas, allows us to re-examine the relative importance of selective abortion in stabilizing pollination–seed consumption mutualisms. We can now ask the question: what general characteristics of pollination system make them more or less likely to employ this type of mechanism? *Breynia* spp. and *Phyllanthus* spp., both close relatives of *Glochidion* and both also pollinated by *Epicephala* moths, are not likely to employ selective abortion as a mechanism to avoid exploitation (Kawakita and Kato 2004a,b). A major difference in these systems is the mechanics of the oviposition. In *Breynia* and *Phyllanthus*, *Epicephala* moths oviposit on the surface of the ovary, whereas moths pollinating *Glochidion* pierce through floral tissue with their ovipositors. If, as suggested in yuccas (Wilson & Addicott 1998; Addicott & Bao 1999; Marr & Pellmyr 2003), internal oviposition is the primary cause of selective abortion, it is possible that selective abortion is a common mechanism limiting the cost of seed predation in the > 300 species of *Glochidion*, and that some other mechanisms are functioning in species of *Breynia* and *Phyllanthus*.

Although further studies are needed to evaluate other potential factors that limit the cost of mutualism, research from less-explored, newly discovered mutualisms is widening our scope. Comparative analyses across different systems will build a more complete understanding of the factors that contribute to long-term persistence of pollination–seed consumption mutualisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article

Table S1 Proportions of flowers with zero to four eggs in retained/aborted flowers.
Table S2 Proportions of uneaten seeds in fruits with zero to two eggs.
Appendix S1 Procedures for estimating fitness effects of selective abortion.

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