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Coexistence of mutualists and antagonists: exploring the impact of cheaters on the yucca – yucca moth mutualism

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Abstract Mutualists and non-mutualistic cheaters commonly coexist, but the effect of mutualist-cheater interactions on the evolution and stability of mutualisms or persistence of cheater populations is not well understood. Yuccas and yucca moths are an example of an obligate mutualism in which cheaters are frequently present. Larvae of both pollinators and cheaters eat developing yucca seeds, but cheaters no longer pollinate and rely on the mutualist species for seed availability. In this study we focus on interactions between the cheater *Tegeticula intermedia* and the pollinator *T. yuccasella* in fruits of the host *Yucca filamentosa*. We examined the effect of pollinator and cheater density on larval mass and larval mortality, the effect of fruit mass on larval interactions, and the degree of variability in pollinator-cheater interactions across 3 years. This study was done in a natural population to determine whether these two larval species affect each other under natural conditions. Genetic markers (both protein electrophoresis and DNA sequencing) were used to identify the larvae to species. We found no correlation between larval number and mortality for either pollinators or cheaters. Furthermore, pollinator mass was not correlated with number of cheater larvae per fruit in any year. Mass of cheater larvae was not correlated with number of pollinator larvae per fruit in two of three years; in one year, there was a negative correlation between number of pollinator larvae on mass of cheater larvae only in small fruits. Our results suggest that larval competition between species is weak and asymmetric; when it occurs, cheater larvae incur the cost. The number of cheaters and pollinators per fruit

was negatively correlated in two of three years. The lack of a positive correlation in number of cheater and pollinator larvae per fruit may contribute to lowering the potential for seed competition among larvae. In addition, larval density per fruit differed across years by as much as 30%. However, this degree of difference was apparently not enough to cause seed limitation because no effect of pollinator larvae on either mass or mortality of cheater larvae was detected in years with the highest larval densities per fruit. In contrast to the weak effects of pollinator-cheater interactions, fruit mass accounted for much of the variation in number and mass of both pollinator and cheater larvae within and across years. Larger fruits generally had heavier and higher numbers of larvae compared to smaller fruits. Overall, in this population at least within the time period studied, pollinators and cheaters coexisted with little conflict in fruit. This result is consistent with the hypothesis that the recent rapid radiation of species in the *T. yuccasella* complex may be explained in part by the ability of multiple pollinator species (some of whom have become cheaters) to use fruits without severe competition.

Keywords Mutualism · Antagonism · Species coexistence · Stability of cheaters · Plant-pollinator interactions

Introduction

Mutualisms often attract cheaters that can take advantage of resources without reciprocating. Examples of this type of cheating include fungi that exploit lichen relationships (Richardson 1999), ant species that use resources produced by acacias, but do not protect the tree from herbivores (Janzen 1975; Stanton et al. 1999), non-photosynthetic plants that take advantage of plant-mycorrhizal interactions (Taylor and Bruns 1997), and nectar thieves or seed predators that take floral resources, but do not pollinate in return (Inouye 1980; Bronstein 1991; Kerdelhue and Rasplus 1996; Pellmyr et al. 1996; Herre and West

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1997; Irwin and Brody 1999). Although mutualists and cheaters commonly coexist, the impact of cheaters on mutualist partners is not well understood.

Cheaters may appear within a mutualism either by an invasion of a third species or by evolving from a mutualist, in other words, a reversal of mutualism. In yucca moths, there is evidence of two separate reversals of mutualism, with cheater species now coexisting with pollinators (Pellmyr et al. 1996; Pellmyr and Leebens-Mack 2000). The cheaters no longer pollinate, but their larvae consume yucca seeds, and they rely upon other species to pollinate yucca flowers. Cheaters coexist with at least 8 of the 11 pollinator species that have been described within the *Tegeticula yuccasella* complex (Pellmyr et al. 1996; Pellmyr 1999; Pellmyr and Leebens-Mack 1999). Based on an analysis of the rate of genetic divergence, *Tegeticula* pollinators have been estimated to be between 35 million and 41 million years old, whereas cheater lineages date to 1.25 ± 0.9 million years old (Pellmyr and Leebens-Mack 1999). The difference in age between pollinator and cheater lineages raises questions regarding whether conditions required for the evolution of cheating rarely occur, or whether cheaters commonly arise, but only a small fraction persist.

The persistence of cheating is likely to be affected by the strength of pollinator-cheater interactions (McPeck 1996; Young et al. 1997). In populations of *Yucca filamentosa*, the pollinator, *T. yuccasella*, coexists with the cheater, *T. intermedia*. Differences in timing of oviposition limit the amount of direct interaction between adults of cheaters and pollinators; however, there is potential for significant interactions among larvae. Larvae of both moth species only feed on developing yucca seeds and overlap in feeding time. Pollinator eggs are laid earlier and larvae begin feeding up to a week earlier than cheater larvae such that when a first-instar cheater larva enters the fruit it is encountering third- or fourth-instar pollinator larvae. Therefore, competition between cheater and pollinator larvae could occur either directly through predation, or indirectly through resource-limited competition if seeds are limiting. In either case, one would expect asymmetric competition due to the smaller size of cheaters (Denno et al. 1995). Another possible outcome is that cheater and pollinator larvae may have limited interactions with minimal effects on each other. We conducted a 3-year study to address three questions directly related to the issue of mutualist-cheater coexistence. (1) How do cheater larvae impact the mass and mortality of pollinator larvae and vice versa? (2) Do plant traits, such as fruit mass, affect pollinator-cheater interactions? (3) Are there differences among years in pollinator-cheater interactions?

Materials and methods

Study species

In the population used in this study there are two *Tegeticula* species (Lepidoptera: Prodoxidae) that consume the seeds of *Y. fila-*

mentosa as their sole food resource. *Tegeticula yuccasella* (Riley) oviposits into first-day flowers and serves as the only local pollinator of *Y. filamentosa*. Moths become active around dusk and remain active for 2–4 h. After a female has collected pollen, she oviposits into the ovary placing an egg next to the ovules. The female then uses her maxillary tentacles to take some of the pollen from her batch and pollinate the flower. A female may oviposit into and pollinate a flower several times before moving to another flower to repeat the procedure. Pollinator eggs are laid directly in the locule, thus once an egg hatches approximately 7 days after being laid, the larva can immediately begin feeding on seeds. The larva feeds for 3–4 weeks, then exits through the fruit wall, burrows into the ground, and overwinters in a cocoon in the soil. In a sample of 180 fruits containing only pollinator larvae, each pollinator larva ate on average 21 ± 4.1 ($x \pm 1$ SD) seeds (Pellmyr et al. 1996).

The cheater *T. intermedia* (Riley) oviposits into young fruit. Eggs are laid one at a time superficially inside the wall of 6- to 20-day-old fruits (Pellmyr 1999). The larva hatches in less than 5 days, chews its way into the locular cavity and begins feeding on seeds. Based on the timing of cheater oviposition and egg hatching, cheater larvae begin feeding on seeds 5–15 days after pollinator larvae have begun feeding on seeds. The cheater larva also exits through the fruit wall and overwinters in a cocoon in the soil. In a sample of 66 fruits containing only cheater larvae, each cheater larva ate on average 26.5 ± 9.8 ($x \pm 1$ SD) seeds (Huth and Pellmyr, unpublished data).

The host plant, *Y. filamentosa* L. (Agavaceae), is a perennial native to coastal areas of the southeastern United States, and since the 1800s has been introduced throughout the eastern United States by settlers (Riley 1892; Trelease 1902; Gleason and Cronquist 1991). A 1- to 2-m-tall paniculate inflorescence with 100–475 flowers is produced from a basal rosette of leaves. Flowers open in the evening; anthers dehisce just before dusk, and stigmas are receptive upon flower opening. Flowers on an inflorescence open over the course of 10–20 days, and each flower is receptive to pollen for 1–2 days, but pollinators usually reject second-day flowers. The capsular fruits mature and dehisce 6–8 weeks after pollination, producing up to 300 seeds per fruit. Pollinator and cheater larvae emerge from fruit before the fruit dehisces, and most larvae emerge within a 1- to 2-week period (Groman and Pellmyr, unpublished data).

Field site and data collection

Pollinator-cheater interactions were studied for 3 years in a *Y. filamentosa* population located in a secluded cedar glade in central Tennessee, USA (Rutherford County, N $36^{\circ}02'$, W $86^{\circ}24'$). The glade had approximately 160 *Y. filamentosa* with 50–70 flowering individuals each year. Each year all flowering individuals were mapped and numbered, and 20 plants were chosen using a random numbers table. Up to 10 fruit were collected from each of these flowering stalks shortly before larvae emerged from the fruit. Larvae were extracted from fruit and frozen at -80°C . Fruit mass, number of pollinator and cheater larvae per fruit, and weight of each larva was determined each year.

An estimate of larval mortality was made in 1996 and 1998. Because oviposition behavior differs between the two species, the measurement of mortality is slightly different for pollinators and cheaters. Pollinator ovipositors pierce through the ovary wall, which leaves a characteristic teardrop to oval-shaped scar on the inside of the ovary wall that can be reliably counted. Pollinator mortality was calculated as $1 - (\text{number of pollinator larvae per number of pollinator ovipositions})$. Pollinators fail to lay an egg on average 30% of the time they attempt to oviposit (Pellmyr and Huth 1994); thus our measurement of pollinator mortality overestimates the amount of mortality occurring between egg and later instar stages. Cheater oviposition scars leave a superficial bump on the outside ovary wall and are more difficult to count reliably. However, cheater larvae make entry holes into the locule that can be unambiguously counted. Entry holes provide an estimate of the number of first instar cheater larvae that enter the fruit before

beginning to feed on seeds. Cheater mortality was calculated as $1 - (\text{number of cheater larvae per total number of first instar cheater larvae entering the fruit})$. The difference in measurement of mortality between egg to late instar would bias the results toward overestimating the amount of pollinator mortality and underestimating the amount of cheater mortality if many cheaters die between egg and first instar.

Identifying pollinator and cheater larvae

Basic information about the interaction between pollinator and cheater larvae is lacking, in part, because there are no diagnostic morphological markers that distinguish the larvae. Adult cheaters and pollinators, which can be unambiguously identified, were used to screen for species-specific genetic markers. Six isozymes (GOT, IDH, HK, LAP, PGM, and PGI) were screened to identify systems that had potential as genetic markers in 19 adult cheaters and pollinators. Although several isozymes were polymorphic, only one of the six isozymes, GOT, had unique bands for cheaters and pollinators. The utility of GOT for providing reliable markers of *T. yuccasella* and *T. intermedia* larvae was tested and confirmed by using diagnostic mtDNA sequence variation. Thirty-one larvae and four adults were cut in half for both enzyme and DNA extraction. Enzyme extracts were run on starch gels, and DNA extracts were sequenced using the methods described below.

Once the utility of the GOT marker was confirmed, protein electrophoresis was used on all larvae heavier than 0.005 g; <8% of the larvae weighed less than 0.005 g. For allozyme analysis, electrophoretic extracts were made by grinding larvae in 0.2 M TRIS-HCl, pH 8.0 buffer and beta-mercaptoethanol. Starch gels were made with 12% potato starch in a 1:10 dilution of the electrode buffer, TRIS-maleate-EDTA pH 7.4 (Werth 1985). The stain for GOT was modified from Werth (1985) in that we used 0.2 M TRIS HCl pH 8.0. Gels were run at 100 mA until the proteins had migrated 3 cm, taking approximately 8 h. The informative locus was GOT-1 (slow locus); cheaters were homozygous for the slow allele and pollinators were homozygous for the fast allele.

DNA sequencing was used for samples that gave ambiguous isozyme banding patterns and for larvae that were too small to extract enough enzyme (<0.005 g). DNA extraction protocols followed Harrison et al. (1987) for extracting DNA from whole larvae. A modification of Hillis et al. (1996) was used for extracting DNA from larvae that had been crushed in the isozyme grinding buffer. Modifications included adding 250 μ l of STE buffer, pH 8.0 to the larval homogenate, instead of proteinase K, 10% SDS, and STE buffer. DNA was extracted immediately from the homogenate with 100 μ l of CHCl_3 : isoamyl alcohol (41:1) (PCI mixture) and 450 μ l of phenol. In the second re-extraction 500 μ l of (24:1) chloroform: isoamyl alcohol was used rather than repeating the extraction with the PCI mixture listed above. DNA sequencing data were based on a 507-bp region of cytochrome oxidase I in mtDNA positions 2,240–2,760 in the *Drosophila yakuba* genome; Clary and Wolstenholme 1985). The region was PCR amplified and one strand was sequenced with Amersham Dye-terminator chemistry on an ABI 377 automated sequencer following the manufacturer's protocols (Amersham Pharmacia). Known sequences of the pollinator *T. yuccasella* (Genbank accession no. U49032) and cheater *T. intermedia* (Genbank accession no. U49030) were used in a neighbor-joining analysis in PAUP. There is ~2% sequence divergence between cheaters and pollinators in the region used (Pellmyr and Leebens-Mack 1999), and samples fell into two distinct groups of larvae representing either *T. yuccasella* or *T. intermedia*. We weighed and identified a total of 870 larvae in 162 fruits in 1996, 1,052 larvae in 139 fruits in 1997, and 1,025 larvae in 181 fruits in 1998.

Statistical analysis

Yearly variation in fruit mass, larval number, and larval mass was analyzed using Welch's ANOVA, which does not assume homogeneity of variance (Zar 1996). Contrasts were performed using

Tukey-Kramer multiple comparison (Zar 1996). The effect of within-year fruit mass variation on larval number, larval mass, oviposition, and mortality was analyzed using Spearman's rank correlations. The correlation between number of pollinators and number of cheaters per fruit was measured using Spearman's rank correlations, and a Kruskal-Wallis chi-square test was used to test differences among years on the proportion of cheaters per fruit. Proportions were arcsine square-root transformed for the analysis to meet the assumption of normality. Chi-square analysis was used to test differences among years on the proportion of fruits containing both cheater and pollinator larvae.

Plant characteristics other than fruit mass, such as location and timing of flowering, had significant effects on larval number and larval mass. Therefore, to determine whether the number of pollinator larvae affected mass of cheater larvae, we statistically controlled for plant effects by including plant as a random factor and fruit mass as a covariate in an analysis of covariance (ANCOVA). Altogether the model included the response variables, larval mass, fruit mass and larval number, as covariates, and plant was considered a random factor (also see West and Herre 1994). Each year was analyzed separately. ANCOVA was also used to analyze the effect of larval number on larval mortality. Plant, fruit mass, and larval number were included in the model as described above. All analyses were performed using JMP, version 3.2 (SAS Institute 1994).

Results

Effect of fruit mass on pollinators and cheaters

Both the number and mass of larvae per fruit were significantly affected by fruit mass among and within years (Fig. 1, Table 1). Fruit mass differed significantly among years, with a threefold difference among years (Fig. 1a); the lowest average fruit mass occurred in 1998. Pollinator and cheater larval mass also differed significantly among years (Fig. 1b) with lowest average pollinator and cheater larval mass occurring in 1998. Within years, the number of pollinator and cheater larvae was positively correlated with fruit mass (Table 1). Mass of pollinator larvae was also positively correlated with fruit mass in all years, whereas mass of cheater larvae was significantly correlated in two of the three years of study.

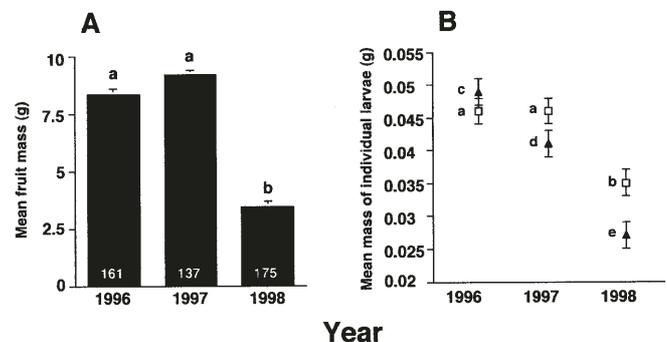


Fig. 1 A Mean (\pm SE) per year of fruit mass, and B the mean mass of individual larvae. (Fruit mass Welch ANOVA $F=233.73$, 2 *df*, $P<0.0001$; pollinator mass $F=24.74$, 2 *df*, $P<0.0001$; cheater mass $F=33.72$, 2 *df*, $P<0.0001$). Number of fruit sampled is indicated on the bars in A. Significant differences among years are indicated by different letters; in B contrasts for pollinator mass are indicated by letters *a* and *b*, and for cheater mass by letters *c*, *d*, and *e*. ■ Pollinator larvae ▲ cheater larvae

Table 1 Correlations between fruit mass and larval mass, and fruit mass and larval number. See Fig. 1 for mean \pm 1 SE of fruit mass, pollinator mass, and cheater mass. Significance levels are indicated by *; $P < 0.025$ and $P < 0.0001$

Larval mass	Range in larval mass per fruit (g)	No. of fruits	Spearman r_s
1996			
Pollinator mass \times fruit mass	0.003–0.08	161	+0.53***
Cheater mass \times fruit mass	0.007–0.07		+0.65***
1997			
Pollinator mass \times fruit mass	0.013–0.07	137	+0.53***
Cheater mass \times fruit mass	0.002–0.07		+0.29**
1998			
Pollinator mass \times fruit mass	0.001–0.08	175	+0.49***
Cheater mass \times fruit mass	0.001–0.05		+0.19
Larval number	Range in larval number per fruit		Spearman r_s
1996			
Pollinator no. \times fruit mass	0–14	162	+0.17***
Cheater no. \times fruit mass	0–18		+0.24***
1997			
Pollinator no. \times fruit mass	0–19	139	+0.41***
Cheater no. \times fruit mass	0–20		+0.51***
1998			
Pollinator no. \times fruit mass	0–15	181	+0.42***
Cheater no. \times fruit mass	0–11		+0.26***

Table 2 Summary of pollinator and cheater oviposition and mortality. Mean (\pm 1 SE)

Year	Number of pollinator ovipositions per fruit	Percent pollinators died as eggs or early instars	Number of cheater larvae entering fruit	Percent cheaters died before entering fruit
1996	10.24 (0.4)	59.6 (2.5)	5.04 (0.7)	64.4 (4.0)
1998	8.08 (0.3)	43.3 (2.2)	2.56 (0.3)	62.1 (0.04)

Table 3 Correlation between fruit mass and number of ovipositions or larval mortality. Significance level is indicated by *; $P < 0.0002$

Year	Spearman r_s
1996	
Pollinator oviposition \times fruit mass	-0.02
Cheater oviposition \times fruit mass	-0.01
Pollinator mortality \times fruit mass	-0.01
Cheater mortality \times fruit mass	-0.06
1998	
Pollinator oviposition \times fruit mass	+0.41***
Cheater oviposition \times fruit mass	+0.31***
Pollinator mortality \times fruit mass	-0.28***
Cheater mortality \times fruit mass	-0.06

The effect of fruit mass on oviposition number and larval mortality varied between years (Tables 2, 3). The number of pollinator and cheater ovipositions were not correlated with fruit mass in 1996, but in 1998 the number of pollinator and cheater ovipositions was significantly greater in larger fruits compared to smaller fruits. In terms of larval mortality, the only significant effect was one of greater mortality of pollinators in small fruits in 1998.

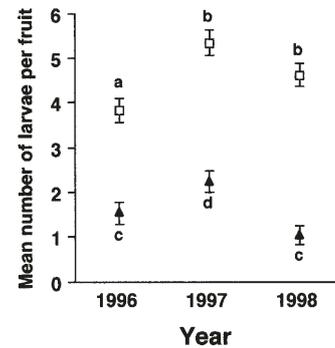


Fig. 2 Mean number of larvae per fruit (\pm 1 SE) per year pollinators, Welch ANOVA $F=4.96$, 2 df , $P=0.008$; cheaters, $F=3.64$, 2 df , $P=0.03$). Significant differences among years are indicated by different letters. ■ Pollinator larvae ▲ cheater larvae

Interaction between pollinators and cheaters

The density of pollinator and cheater larvae per fruit differed across years (Fig. 2). Although there was yearly variation in number of larvae, the proportion of cheaters and pollinators was similar across years. On average, cheaters comprised 25% of total number of larvae, and the proportion of cheater larvae to all larvae did not differ significantly across years 1996=28.6%, 1997=29.5%, 1998=18.4%; Kruskal-Wallis=4.49, 2 df ,

Table 4 Analysis of covariance analyzing effect of plant, fruit mass, and number of larvae on larval mass. Bold *P* values indicate years in which number of larvae per fruit had an effect on larval mass.

Year	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
1996 mass of cheater larvae				
Number of pollinator larvae per fruit	1	0.00064	5.51	0.023
Fruit mass × number of pollinators	1	0.00047	4.02	0.051
Fruit mass	1	0.000004	0.03	0.861
Plant	14	0.0003	2.55	0.009
Model	17	0.00028	2.39	0.010
Error	44	0.00012		
1996 mass of pollinator larvae				
Number of cheater larvae per fruit	1	0.00005	0.59	0.443
Fruit mass × number of cheaters	1	0.00019	2.11	0.149
Fruit mass	1	0.00301	33.89	0.0001
Plant	17	0.00021	2.40	0.003
Model	20	0.00039	4.37	0.0001
Error	124	0.00009		
1997 mass of cheater larvae				
Number of pollinator larvae per fruit	1	0.00001	0.13	0.725
Fruit mass × number of pollinators	1	0.00008	1.11	0.302
Fruit mass	1	0.00010	1.51	0.230
Plant	13	0.00034	4.94	0.0002
Model	16	0.00048	6.96	0.0001
Error	28	0.00007		
1997 mass of pollinator larvae				
Number of cheater larvae per fruit	1	0.00019	4.05	0.047
Fruit mass × number of cheaters	1	0.00024	5.099	0.027
Fruit mass	1	0.00066	13.92	0.0003
Plant	14	0.00026	5.46	0.0001
Model	17	0.00036	7.55	0.0001
Error	84	0.00005		
1998 mass of cheater larvae				
Number of pollinator larvae per fruit	1	0.00018	0.82	0.37
Fruit mass × number of pollinators	1	0.00060	2.65	0.11
Fruit mass	1	0.00047	2.066	0.16
Plant	17	0.00045	2.01	0.03
Model	20	0.00049	2.17	0.02
Error	46	0.00023		
1998 mass of pollinator larvae				
Number of cheater larvae per fruit	1	0.00032	1.91	0.17
Fruit mass × number of cheaters	1	0.0000002	0.0011	0.97
Fruit mass	1	0.00248	14.66	0.0002
Plant	19	0.00091	5.38	0.0001
Model	22	0.00126	7.43	0.0001
Error	148	0.00017		

$P=0.11$). The mean proportion of fruits containing both cheaters and pollinators was 34% and did not differ significantly across years (1996=29%, 1997=36%, 1998=36%; chi-square =2.88, 2 *df*, $P=0.24$).

Number of cheater larvae per fruit was negatively correlated with number of pollinator larvae in two of three years (Fig. 3; Spearman's r_s for 1996 $r_s = -0.38$, $P < 0.0001$, $N=168$ fruits; 1997 $r_s = -0.23$, $P=0.006$, $n=149$ fruits; 1998 $r_s = +0.11$, $P=0.15$, $n=181$ fruits). The correlations remained significant when fruit mass and plant were included in more complicated models.

We explored the potential effect of pollinator-cheater interactions using two measures: larval mass and larval mortality. In two of three years, number of pollinator larvae had no significant impact on mass of cheater larvae (Table 4). In 1996, pollinator number had a signif-

icant negative effect on cheater mass, and the interaction between pollinator number and fruit mass was marginally significant (Table 4). Further analysis of the 1996 data was done in which the data were categorized into small fruits (=8.0 g) and large fruits (=8.0 g), based on the distribution of fruit mass (mean and median =8 g). This showed that cheater mass was negatively affected by pollinator number in only small fruits, and that there was no effect of pollinator number on cheater mass in fruits larger than 8.0 g (Table 5).

There were no cases in which number of cheater larvae had a negative impact on pollinator mass (Table 4). In two of three years there was no significant effect of number of cheater larvae per fruit on mass of pollinator larvae. In one year, 1997, pollinator larvae were heavier in fruit that had higher numbers of cheater

Table 5 Analysis of 1996 data for effect of pollinator number on cheater mass in small and large fruits. Based on the distribution of fruit mass, data were divided into two groups: small fruits were categorized as less than 8.0 g and large fruits were categorized as greater than 8.0 g. Bold *P* values indicate a significant effect of larval number on larval mass

	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Small fruits – 1996				
Mass of cheater larvae				
Number of pollinator larvae per fruit	1	0.00107	9.04	0.008
Fruit mass × number of pollinators	1	0.00093	7.87	0.012
Fruit mass	1	0.00017	1.43	0.248
Plant	9	0.00019	1.65	0.180
Model	12	0.00030	2.59	0.036
Error	17	0.00012		
Large fruits – 1996				
Mass of cheater larvae				
Number of pollinator larvae per fruit	1	0.00007	1.07	0.320
Fruit mass × number of pollinators	1	0.00004	0.56	0.466
Fruit mass	1	0.00030	4.79	0.047
Plant	14	0.00016	2.52	0.053
Model	17	0.00018	2.76	0.035
Error	13	0.00006		

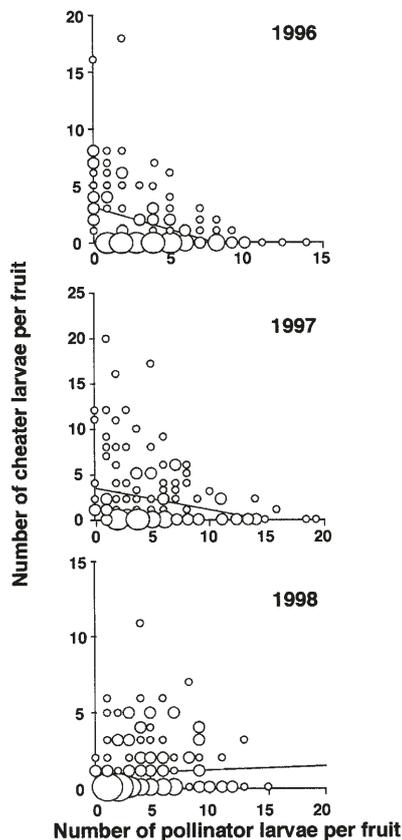


Fig. 3 Correlation between number of cheater and pollinator larvae per fruit. Lines from linear regression are shown. Linear regression equations are 1996 $Y=3.0-0.39X$, adjusted $r^2=0.14$; 1997 $Y=3.5-0.24X$, adjusted $r^2=0.06$; 1998 $Y=0.92+0.03X$, adjusted $r^2=-0.003$. Circles of five different sizes (1–5, from smallest to largest) indicate the number of observations: 1 1, 2 2–5, 3 6–10, 4 11–20, 5 21–25

larvae, and there was a significant interaction between fruit mass and number of cheaters. Similarly, larval mortality was not affected by coexisting in the same fruit with larvae of the other species (Fig. 4, Table 6). There was no significant effect of either cheater number on

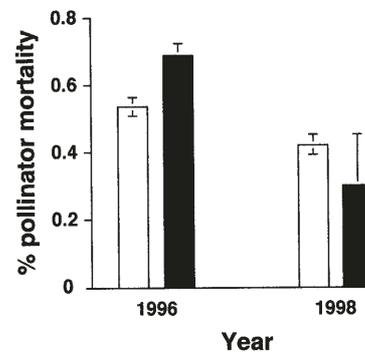


Fig. 4 Proportion of pollinator mortality (± 1 SE) in fruits with and without cheaters for 1996 and 1998. □ No cheater larvae in fruit ■ cheater larvae present in fruit

pollinator mortality or pollinator number on cheater mortality in either 1996 or 1998.

Discussion

Cheater and pollinator larvae can potentially interact indirectly through competition for seeds, directly through predation, or coexist with little impact on each other. Three lines of evidence suggest that pollinators and cheaters seldom compete for seeds, at least as measured in this study. First, pollinator mass was never affected by number of cheater larvae per fruit, and cheater mass was unaffected by number of pollinators in two of three years. In one year, there was a negative effect of pollinator number on cheater mass only in small fruits, although compared to fruit mass and plant effects, pollinator number had a small effect on cheater mass. Second, larvae did not appear to be limited by seed availability. Larval mortality was generally not affected by fruit mass. Cheater mortality was not correlated with fruit mass. Pollinator mortality was greater in small fruits only in 1998, although intact seeds were found in 97% of the fruit in 1998 suggesting that higher mortality was not

Table 6 Analysis of covariance for effect of plant, fruit mass, and larval number on larval mortality. Effect of cheater number on pollinator mortality

Year	Terms	df	MS	F	P
1996	Number of cheaters	1	0.215	3.10	0.08
	Fruit mass × cheater no.	1	0.074	1.06	0.30
	Fruit mass	1	0.279	4.02	0.05
	Plant	17	0.263	3.78	0.0001
	Model	20	0.393	5.65	0.0001
	Error	136	0.069		
1998	Number of cheaters	1	0.082	1.15	0.29
	Fruit mass × cheater no.	1	0.0183	0.26	0.61
	Fruit mass	1	0.005	0.07	0.79
	Plant	17	0.100	1.41	0.15
	Model	20	0.111	1.56	0.08
	error	82	0.071		
Effect of pollinator number on cheater mortality					
1996	Number of pollinators	1	0.355	1.83	0.18
	Fruit mass × pollinator no.	1	0.124	0.64	0.43
	Fruit mass	1	0.023	0.12	0.73
	Plant	15	0.153	0.79	0.68
	Model	18	0.321	1.67	0.07
	Error	63	0.194		
1998	Number of pollinators	1	0.023	0.26	0.61
	Fruit mass × pollinator no.	1	0.084	0.95	0.34
	Fruit mass	1	0.047	0.53	0.48
	Plant	17	0.098	1.11	0.41
	Model	20		1.17	0.36
	Error	20			

explained by lack of seeds. Intact seeds are commonly found in yucca fruits; in a study of eight species of yucca in which some of the populations most likely contained both pollinators and cheaters, the average proportion of seeds eaten per fruit ranged from 0.6% to 35% (Addicott 1986). In the present study, there were differences across years in larval density per fruit. For example, there were 30% more larvae per fruit in 1997 compared to 1996 and 1998; however, this increase in larval density did not significantly affect the mass of pollinator and cheater larvae most likely because seeds were not limiting. Third, the negative correlation between number of cheaters and pollinators per fruit in two of three years suggests that cheaters discriminate against ovipositing into fruits with many pollinator larvae, or alternatively, that some plants may possess traits that disproportionately affect cheaters. The tendency for high numbers of cheaters to occur in fruits with low numbers of pollinators further decreases the potential for seed competition among pollinators and cheaters. The fact that the negative correlation occurs in some years, but not in others, indicates that other factors may override either cheater oviposition choice or a plant's ability to discriminate against cheaters. In a related study of oviposition choice by two distinctive pollinator species co-occurring on *Y. kanabensis*, slight asymmetry in avoiding previously visited flowers was documented. One species did not alter its oviposition behavior in response to the other, whereas the other species tended to avoid flowers with eggs of the first species, but laid more eggs per flower when it did oviposit into flowers containing eggs of the first species (Wilson and

Addicott 1998). In addition, in our study there was no evidence for predation between pollinators and cheaters as measured through the effect of larval number on mortality. Overall, pollinators and cheaters had a negligible impact on each other in terms of number of larvae per fruit, larval mass, and larval mortality.

In contrast to the weak effects of pollinator-cheater interactions, fruit mass accounted for much of the variation in number and mass of both pollinator and cheater larvae within and across years. Within years, larger fruits generally had heavier and higher numbers of larvae compared to smaller fruits. Yearly differences in larval mass followed the pattern of variation in fruit mass (compare Fig. 1A, B). For example, larval mass of both pollinators and cheaters was lowest in 1998, which corresponded with low fruit mass in 1998. Weather-related factors are likely to have contributed to the yearly variation in fruit mass. Low rainfall the preceding fall may have contributed to low 1998 fruit mass; average rainfall from July to December 1997 was 35% lower compared to the same time period in 1996, and 20% lower compared to 1995 (National Climatic Data Center 2000). The size of phytophagous insects, particularly those that are limited to feeding on a single plant, is commonly associated with the quantity and quality of available plant tissue (reviews in Slansky 1993; Price 1997). For example, in figs a greater proportion of variation in wasp body size is explained by the fruit that the wasp is born in compared to the size of the maternal wasp (Herre 1989). In yuccas, larval mass was greater in larger fruits perhaps reflecting differences in seed quality between small and large

fruits, because seed number was generally not a limiting factor even in small fruits. Both pollinator and cheater larvae seem to benefit from being in large fruit, yet larger fruits did not have significantly more ovipositions than small fruits in all years. Oviposition was correlated with fruit size only in 1998. The unusually low fruit mass of 1998 may have increased the degree of discrimination against ovipositing in very small flowers, however, more work is needed examining factors that affect oviposition choice.

The potential for species coexistence may increase when species interactions are weak because weak interactions provide little selective force on species (McPeck 1996). The consistency in proportion of fruits with cheaters and pollinators across years, as well as limited effect of pollinators and cheaters on their number, mass, and mortality shows that these two species share fruit resources with minimal impact on each other in terms of larval success. This outcome may not hold true for all populations. Given the constraints of pollinator and cheater biology, we consider how the outcome of pollinator-cheater interactions may vary over time or space. The number of pollinator eggs that can be laid is limited by selective floral abscission (Pellmyr and Huth 1994), which effectively limits pollinator seed consumption to an average of 26% seeds per fruit (Pellmyr et al. 1996). In effect, pollinator larvae cannot reach high levels of density per fruit. In contrast, the number of cheaters per fruit is not limited by selective abscission, and in some populations cheaters can reach high enough densities that all seeds are destroyed in *Y. filamentosa* fruit (Pellmyr et al. 1996). Variation in seed consumption both among fruits and across years, ranging from 0 to 100% seed loss, has also been reported in other yucca species (Keeley et al. 1984; Addicott 1986). In years of high cheater density per fruit, seeds will become limiting, but the early oviposition of pollinators and their head start in feeding would likely result in asymmetric competition with cheaters incurring a higher cost than pollinators in seed-limiting conditions. The negative effect of increasing pollinator number on cheater larval mass, and no effect of cheater number on pollinator mass, in small fruits in 1996 supports the idea that competition is asymmetric when it occurs. Although experimental manipulation of larval densities might demonstrate that competition is asymmetric, the results presented here suggest that such densities are seldom realized in nature.

Interactions among species in other seed-parasite pollinator mutualisms

In addition to the yucca/yucca moth system, there are two other seed-parasite pollination mutualisms, fig/fig wasp and globeflower/globeflower fly, where the pollinator coexists with multiple species feeding on seeds in the fruit. Fig fruits host a complex community of both pollinators and non-pollinators such as the gallmakers (Janzen 1979; Bronstein 1991; Compton et al. 1994;

Kerdelhue and Rasplus 1996; Machado et al. 1996; Herre 1999). Both pollinators and gallmakers prefer short-style flowers, and there is competition for oviposition sites, but the impact of gallmakers on fig pollinators depends on the species of gallmaker (West and Herre 1994; Kerdelhue and Rasplus 1996). Early-ovipositing gallmakers, in the genera *Idarnes* and *Sycophaga*, have a negative impact on the number of pollinator wasps emerging from fruit because they directly compete with pollinators for oviposition sites (West and Herre 1994; Kerdelhue and Rasplus 1996; Herre 1999). Late-ovipositing gallmakers such as *Apocryptophagus* sp. have little impact on pollinators because pollinators are well-established in the fig fruit by the time a late gallmaker oviposits (Kerdelhue and Rasplus 1996).

Fig pollinator-gallmaker interactions differ in at least three ways from the yucca system. First, in figs, wasps that delay oviposition in figs have fewer oviposition sites available to them, whereas in yuccas, cheaters and pollinators do not compete for oviposition sites. In fact, delaying oviposition may benefit cheaters by reducing the risk of losing offspring to floral abscission (Pellmyr and Leebens-Mack 2000). Delaying oviposition could potentially result in asymmetric competition between larvae of pollinators and cheaters, however, based on the data from the present study, cheater larvae were not limited in access to seeds. Second, the impact of non-pollinators on seed production differs between figs and yuccas. Fig gallmakers appear to have minimal effect on fig seed production, at least as measured by direct loss of seeds. In contrast, cheater yucca moths increase the cost of seed loss. Third, cheater yucca moths can only develop in pollinated fruit; therefore, they must coexist with yucca pollinators. In contrast, *Idarnes* and *Sycophaga* wasps can develop in unpollinated fruit, thus gallmakers do not depend on the pollination services of female pollinator wasps (Bronstein 1991; Compton et al. 1994; West and Herre 1994; Kerdelhue and Rasplus 1996). Some gallmakers depend upon pollinator males to chew an exit tunnel in order to escape the fig (Kerdelhue and Rasplus 1996), but *Idarnes* sp. have been observed in high numbers in unpollinated figs so some gallmakers may be able to exist independent of pollinator wasps (Bronstein 1991; West and Herre 1994). In summary, the outcome of the interaction between gallmakers and fig pollinators is greatly affected by timing of oviposition. Early gallmakers have the greatest potential to negatively affect the population dynamics of fig pollinators by usurping oviposition sites, whereas late gallmakers apparently have little impact on the population dynamics of pollinators.

The globeflower/globeflower fly system differs from yuccas and figs in that up to six different pollinator species (*Chiastocheta* spp.) co-occur in populations of *Trollius europaeus*; in the yucca and fig system there are typically 1 or 2 pollinator species in a population. One study examining interspecific competition among fly species in the French Alps showed no evidence for predation among *Chiastocheta* larvae (Jaeger et al. 2001). There was a decrease in seed consumption per larva in fruits

with higher egg loads, which was interpreted as evidence for competition among larvae for resources (Jaeger et al. 2001). There was never complete seed destruction even in fruits with high larval densities. Each fly species oviposits at a different time, but it is not known whether competition was asymmetric between early and late ovipositing pollinators because larvae could not be identified to species. While the outcomes of species interactions in yuccas, figs, and globeflowers differ in their impact on the plants and pollinators, perhaps the common thread among these systems is the remarkable resilience of the mutualism despite multiple demands on fruit resources.

In conclusion, the stability of mutualisms depends upon the costs and benefits of the interaction, which can vary in time and space due to interactions with a third species (Bronstein 1994; Gaume et al. 1998). In the case of *Y. filamentosa* and *T. yuccasella*, the presence of the cheater *T. intermedia* increases seed costs for the plant, but appears to have little direct benefit or cost to the pollinator. The number of larvae per fruit and larval mass differed significantly across years, but numbers of pollinator and cheater larvae did not reach the threshold presumably required to cause competition for seeds. Any effects of cheaters on the pollinator would have to be indirect by limiting production of viable seeds and ultimately reducing the plant population through lower seedling recruitment. Overall, the results of this study show that cheaters and pollinators can coexist with little conflict in fruit. This result is consistent with the hypothesis that the recent rapid radiation of species within the *T. yuccasella* complex may be explained in part by the ability of multiple pollinator species (some of whom have become cheaters) to use fruits without severe competition (Pellmyr and Leebens-Mack 2000). Our work also raises the question of what regulates cheater populations. Asymmetric competition, which is more likely to occur at high cheater densities, is one factor that may limit cheater population growth. Future work on the factors regulating the density of cheater populations and assessing the variability of cheaters across populations will increase our understanding of how cheaters evolve and persist.

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References

- Addicott JF (1986) Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. *Oecologia* 70:486-494
- Bronstein JL (1991) The nonpollinating wasp fauna of *Ficus pertusa*: exploitation of a mutualism? *Oikos* 61:175-186
- Bronstein JL (1994) Conditional outcomes in mutualistic interactions. *Trends Ecol Evol* 9:214-217
- Clary DO, Wolstenholme DR (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *J. Mol Evol* 22:252-271
- Compton SG, Rasplus J, Ware AB (1994) African fig wasp parasitoid communities. In: Hawkins BA, Sheehan W (eds) Parasitoid community ecology. Oxford University Press, Cambridge, Mass., pp 343-370
- Denno RF, McClure MS, Ott JR (1995) Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annu Rev Entomol* 40:297-332
- Gaume L, McKey D, Terrin S (1998) Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant specialist ant and its myrmecophyte host. *Proc R Soc Lond Ser B* 265:569-575
- Gleason HA, Cronquist A (1991) Manual of vascular plants of northeastern United States and adjacent Canada, 2nd edn. New York Botanical Garden, Bronx, N.Y.
- Harrison RG, Rand DM, Wheeler WS (1987) Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Mol Biol Evol* 4:144-158
- Herre EA (1989) Co-evolution of reproductive characteristic in twelve species of new world figs and their pollinator wasps. *Experientia* 45:637-647
- Herre EA (1999) Laws governing species interactions? Encouragement and caution from figs and their associates. In: Keller L (ed) Levels of selection in evolution. Princeton University Press, Princeton, N.J., pp 209-237
- Herre EA, West SA (1997) Conflict of interest in a mutualism: documenting the elusive fig wasp-seed trade-off. *Proc R Soc Lond Ser B* 264:1501-1507
- Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: sequencing and cloning. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics, 2nd edn. Sinauer, Sunderland, Mass.
- Inouye DW (1980) The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia* 45:197-201
- Irwin RE, Brody AK (1999) Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* 80:1703-1712
- Jaeger N, Pompanon F, Despres L (2001) Variation in predation costs with *Chiastocheta* egg number on *Trollius europaeus*: how many seeds to pay for pollination? *Ecol Entomol* 26:56-62
- Janzen DH (1975) *Pseudomyrmex nigropilosa*: a parasite of a mutualism. *Science* 188:936-937
- Janzen DH (1979) How to be a fig. *Annu Rev Ecol Syst* 10:13-51
- Keeley JE, Keeley SC, Swift CC, Lee J (1984) Seed predation due to the Yucca-moth symbiosis. *Am Midl Nat* 112:191-197
- Kerdelhué C, Rasplus J (1996) Non-pollinating Afrotropical fig wasps affect the fig-pollinator mutualism in *Ficus* within the subgenus *Sycomorus*. *Oikos* 75:3-14
- Machado CA, Herre EA, McCafferty S, Bermingham E (1996) Molecular phylogenies of fig pollinating and non-pollinating wasps and the implications for the origins and evolution of the fig-fig wasp mutualism. *J Biogeogr* 23:531-542
- McPeck MA (1996) Linking local species interactions to rates of speciation in communities. *Ecology* 77:1355-1366
- National Climatic Data Center (2000) <http://www.ncdc.noaa.gov/>
- Pellmyr O (1999) Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. *Syst Entomol* 24:1-28
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372:257-260
- Pellmyr O, Leebens-Mack J (1999) Forty million years of mutualism: evidence for an Eocene origin of the yucca-yucca moth association. *Proc Natl Acad Sci USA* 96:9178-9183
- Pellmyr O, Leebens-Mack J (2000) Reversal of mutualism as a mechanism for adaptive radiation in yucca moths. *Am Nat* 156: S62-S76

- Pellmyr O, Leebens-Mack J, Huth CJ (1996) Non-mutualistic yucca moths and their evolutionary consequences. *Nature* 380: 155–156
- Price PW (1997) *Insect ecology*, 3rd edn. Wiley, New York, N.Y.
- Richardson DHS (1999) War in the world of lichens: parasitism and symbiosis as exemplified by lichens and lichenicolous fungi. *Mycol Res* 103:641–650
- Riley CV (1892) Some interrelations of plants and insects. *Proc Biol Soc Wash* 7:81–104
- SAS Institute (1994) *JMP Statistics and graphics guide*, version 3. SAS Institute, Cary, N.C.
- Slansky F (1993) Nutritional ecology: the fundamental quest for nutrients. In: Stamp NE, Casey TM (eds) *Caterpillars: ecological and evolutionary constraints on foraging*. Chapman and Hall, New York, N.Y., pp 29–91
- Stanton ML, Palmer TM, Young TP, Evans A, Turner ML (1999) Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature* 401:578–581
- Taylor DL, Bruns TD (1997) Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. *Proc Natl Acad Sci USA* 94:4510–4515
- Trelease W (1902) *The Yuccae*. Missouri Botanical Garden, Missouri, 13th annual report, pp 27–133
- Werth CR (1985) Implementing an isozyme laboratory at a field station. *Virginia J Sci* 36:53–73
- West SA, Herre EA (1994) The ecology of the New World fig-parasitizing wasps *Idarnes* and implication for the evolution of the fig-pollinator mutualism. *Proc R Soc Lond Ser B* 258: 67–72
- Wilson RD, Addicott JF (1998) Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? *Oikos* 81:109–118
- Young TP, Stubblefield CH, Isbell LA (1997) Ants on swollen-thorn acacias: species coexistence in a simple system. *Oecologia* 109:98–107
- Zar JH (1996) *Biostatistical analysis*, 3rd edn. Prentice Hall, Upper Saddle River, N.J.