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University of Alberta

Partner Regulation in the Mutualism Between Yuccas and Yucca Moths: The Role of Fruit Set Patterns and Surplus Flowers

By

Shelley Ann Humphries ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

In

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ABSTRACT

The obligate pollination/seed predation mutualism between yuccas and yucca moths is a balanced conflict of fitness interests. I investigated two ways that *Yucca kanabensis* McKelvey might regulate its partner to decrease costs in the association. First I documented non-random intrinsic fruit retention patterns favoring flowers from a particular wave and located proximally on inflorescences. I then investigated if non-random fruit retention patterns allowed yuccas to mature fruit of higher quality. I measured seed weight, seed number and seed maturity and larval features such as seed consumption and size. I found no adaptive advantage to yucca flower retention patterns at the level of fruit or larvae. Second, I independently investigated how variation in pollen load, flower position on the inflorescence, and oviposition number affect flower fate. Interplay of these factors allows yuccas to flexibly respond to the pollination environment, developing flowers of highest quality and stabilizing the conflict in this mutualism.

Yucca Frooten

Summertime, the desert wind, the yuccas bloom at night
Moths fly the pollen round, and everything's all right
And the grubs grow strong while the winds blow along
Summertime, the desert wind, the yuccas bloom at night

Millions of years in the making, marvels revealed to our eyes
Moths with odd tentacled faces, and plants reaching up to the skies

Summertime, the desert wind, the yuccas bloom at night
Moths fly the pollen round, and everything's all right
They pollinate, as they cooperate

Out here in the west, a yucca needs its moth
And a moth needs its yucca
That's what makes genuine yucca moths
Such ermine–coated, raven–eyed gems of the desert
They're cute, they're useful-and they gits along with other folks

Whoa, get along little doggie, come visit this bloom and the rest
Roll up a bundle of pollen, and make sure this yucca yucks best

Summertime the desert wind, yuccas bloom at night
Moths fly the pollen round, and everything's all right
And the grubs grow strong while the winds blow along

Summertime, the desert wind, the yuccas bloom at night

lyrics, and permission to
reprint them by John Acorn

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Chapter 1

Conditionality and Regulation in Mutualisms

1.0 INTRODUCTION

1.0.1 Summary

In this chapter I will introduce the basic concept of mutualism and then briefly outline the two main types of mutualism studies done: those that are primarily descriptive and those that are experimental. I will then introduce the concept of conditional outcomes in mutualisms and suggest how this might create situations of instability between mutualistic partners. I will then introduce the concept that stability might be returned to conditional mutualisms if the partners have mechanisms to regulate their costs and benefits. There appear to be three broad mechanisms of regulation that I will summarize with examples. Finally, I will discuss what is known about conditionality and regulation in the context of the mutualism between yuccas and yucca moths, which will lead to the development of the two main questions of my thesis. Do non-random fruit retention patterns in yuccas play a role in the regulation of this mutualism? What are the relative roles of intrinsic plant features and extrinsic moth mediated features on the regulation of this mutualism?

1.0.2 Mutualism

Mutualisms are interspecific interactions between living organisms that reciprocally increase the fitness of participants (Addicott 1995). The term covers a wide range of interactions from obligate or symbiotic situations to those that are more facultative and indirect. It is also common to describe mutualisms in terms of rewards in exchange for service; or in terms of costs and benefits where net benefits to each partner exceed costs (Keeler 1985). Although mutualisms are ubiquitous and ecologically important, we still have only a rudimentary understanding of how inherently selfish behaviors of interacting organisms can evolve and persist in a way that is ultimately mutually beneficial (Frank 1995).

In a recent review, Bronstein (1994a) indicated that most mutualism papers published focus on identifying and describing mutualistic systems (e.g. McLean and Mariscal 1973, Sazima and Sazima 1978, Forester 1979, Stout 1979, Schemske 1980, Marcus 1984, Donaldson 1997). A minority of papers on mutualism are more experimental, attempting to quantitatively document changes in fitness upon the addition or removal of mutualistic partners or resources (e.g. Losey 1979, O'Dowd and Hay 1980, Davidson and Morton 1981, Tomback 1982, Brooks and Gwaltney 1993). From these studies we have learned that mutualisms are very dynamic and that the nature of costs and benefits to partners are not fixed in space and time.

Demonstrating that an interaction is mutually beneficial is challenging and is further complicated by the fact that the outcome of the interaction is not always the same. However, numerous recent papers and reviews have documented the non-static and interesting conditional properties of many mutualistic systems (Abrams 1987, Cushman and Whitham 1989, Breton 1990, Cushman and Addicott 1991, Bronstein 1994b, Setälä *et al.* 1997).

1.0.3 Conditionality

In essence, a mutualism is conditional if the costs and benefits to the participants shift through space and time as a result of changes in abiotic conditions (e.g. soil nutrients) or biotic conditions (e.g. presence or absence of other species or density of mutualists themselves). Conditionality within mutualistic interactions can be influenced by a number of environmental factors and life history patterns (Bronstein 1994a) and conditionality may have important consequences on the dynamics of mutualisms (Cushman and Addicott 1991).

Conditionality seems especially relevant to study if it is capable of destabilizing mutual benefit and shifts the association toward a commensalism. Cushman and Whitham (1989) discuss an example of a conditional mutualism that can shift back and forth between commensalism and mutualism. In this study they showed that ant-tended membracids received no positive benefit from the association in years when predatory spiders were absent. In this way the conditional aspect of the mutualism was influenced by the presence of a third species.

More extreme cases of mutualisms switching to parasitisms can also be found. For example, the mutualism between many plants and their ectomycorrhizal fungi is only beneficial for both partners under low nutrient regimes. If the soil nutrients improve the fungi may actually become parasitic on the plant, (Johnson *et al.* 1997, Setälä *et al.* 1997) acting only as a sink for photosynthate and no longer as a source of nutrients.

Based on the observation that the interactions in many mutualistic pairs are conditional, Bronstein (1994b) has proposed a new format for the (+,+) quadrant of the classic interaction grid to reflect this (Figure 1.1). In the newly devised grid, costs and benefits to species are on a continuum from strongly negative to strongly positive. The positive end for Species 1 corresponds with the negative end for Species 2 with equally balanced costs and benefits for partners occurring somewhere in the middle. This model more accurately reflects the reality of many mutualisms than the simplified original. This model also emphasizes how the interacting species are in conflict because an increasing benefit for one partner can lead to increasing cost for the other. It has been realized for a long time that this conflict of interest can lead to evolutionary instability within cooperative interactions (Axelrod and Hamilton 1981, Bull and Rice 1991). Because of this conditionality many mutualisms may be unstable. However, since mutualisms are so prevalent there must be mechanisms in place that allow partners to respond flexibly and regulate costs and benefits.

1.0.4 Are there examples of regulation within mutualisms?

Do mutualist partners have mechanisms that allow them to adjust or to regulate themselves along the interaction continuum? To answer this question I reviewed a broad sample of papers on mutualism involving a variety of taxa and ranging from obligate symbiosis to facultative associations. I defined regulation as behaviors or properties of mutualist(s) that allow for the modification of the physiology, behavior, or density of its partner(s) resulting in a increase of the benefits relative to the costs of one of its partners. Conditionality seemed to be an important factor for systems that show regulation of partners. Many systems provide optimal benefits for each partner at some given density of partners or under certain environmental conditions. If densities change, or environmental conditions alter, the strength of the mutualism based on costs and benefits also changes. In these systems it appears that methods exist to alter costs and benefits for the partner doing the regulation. Janzen (1985) summarizes all mutualisms using several broad categories including: pollination, seed dispersal, digestive, harvest and protective mutualisms, and examples of evidence for the regulation of mutualistic partners can be found for each of these categories (pollination: Pellmyr and Huth 1994, Schiestl *et al.* 1997; seed dispersal: Murray *et al.* 1994; digestive: Nogge and Ritz 1982; harvest: O'Dowd 1979, Falkowski *et al.* 1993, and protective mutualisms: Pierce *et al.* 1991).

1.0.5 What are the mechanisms of regulation?

I identified at least three different general regulatory mechanisms. The three mechanisms work either by 1) altering partner behavior, 2) altering partner physiology or 3) altering partner density. In the majority of cases it is the host species and not the visitor that is controlling the system. The host is usually the larger partner, the stationary partner, or the partner offering the reward in exchange for service.

1.0.5.1 Behavioral Regulation

The first broad category of regulatory mechanisms are those that function by altering the behavior of mutualist partners. This is done by one partner directing the location or the quality of the reward offered to manipulate the behavior of the other partner to the first partner's improved benefit. For example, some trees can control where, when and how much foliar nectar is produced for defending ants. In this way the plant host can regulate the behavior of the defending ant visitors thus optimally deploying ants at critical times or to sensitive tissues such as flowers or new leaves (O'Dowd 1979). A recent study has also reported similar observations of optimal deployment of ants through the number and location of food bodies produced (Heil *et al.* 1997). Finally, Schiestl *et al.* (1997) have shown that plants can also change the attractiveness of individual flowers to floral visitors - redirecting pollinators to attractive-smelling unpollinated flowers and away from previously pollinated flowers.

1.0.5.2 Physiological Regulation

The next broad category of regulatory mechanisms is where one partner regulates the physiology of another partner for its own benefit. This has been the suggested reason for the observation that compounds in certain bird dispersed fruit act as 'laxative' agents thus regulating the passage rate of the seed in the gut (Murray *et al.* 1994). By influencing gut passage rate the maternal plant can affect the shape of the seed shadow and the viability of the seeds passing through the gut of the dispersal agent. Similar evidence for physiological regulation is shown by bioluminescent squid that directly control symbiont luminescence through controlled oxygen availability to the bacteria (Boettcher *et al.* 1996). This study demonstrated that the squid host has fine control over the luminescent output of the bacteria allowing the squid to remain camouflaged through counter illumination for maximum hunting efficiency.

1.0.5.3 Density Regulation

The third type of regulation is through the manipulation of the number of the mutualists. Mechanisms to both increase and decrease partner density occur. An excellent example of regulation to increase the number of partners is demonstrated by the mutualism between the lycaenid butterfly larvae, *Polyommatus icarus*, and its ant attendant *Lasius flavus*. Many lycaenids are protected from predators and parasitoids by ants and in exchange for the protection the larvae secrete a nutritive reward. Leimar and Axen (1983) experimentally determined that the butterfly larvae behave strategically, attempting to increase the number of ants attending them by increasing production of nutritive secretions (the reward to the ants) when the larvae 'perceive' that they are at risk of predation. Further, it has been shown that parent butterflies are capable of maximizing attendance for their offspring through a number of behaviors including the selection of host plants for high nitrogen content (Pierce *et al.* 1991). Lycaenid larvae also show clumping behaviors which can function to maximize attendance (Pierce *et al.* 1991). The capabilities that these organisms possess to 'fine tune' their mutualistic interactions with ants are remarkable. The one sidedness of these adaptations may also reflect the asymmetry in this mutualism. These interactions are mostly obligate in nature for the host lycaenids but not for the visiting ants. An obligate partner does not have the freedom to defect from the interaction if the exchange is not beneficial and instead must invest in more strategic solutions. Thus it appears that in many systems, an optimum density of partners exists under certain situations and that there is some ability to adjust this level.

Many systems regulate partner density downward toward some optimum threshold and this can be accomplished either indirectly or directly. For indirect regulation, a partner may have its cellular growth or division rate suppressed or modified. This is most common in symbiosis where there is a close physical integration of the partners as in corals (Falkowski *et al.* 1993). All reef corals are mutualistic symbioses between a heterotroph (usually a cnidarian) and a phototroph (dinoflagellate algae, Muscatine and Porter 1977). The density of the symbiont controls the growth of the coral as they provide the carbon that the host needs. It would seem that a host would want as many symbionts as possible to maximize growth; however this is not the case because if symbiont densities become too high they can overgrow the host (Falkowski *et al.* 1993). There are an optimum number of symbionts and for this association to remain stable a mechanism for the regulation of the symbiont must be present. This is accomplished by the cnidarian host secreting a substance that causes the algae to translocate its nitrogen back to the host animal. In this way the algae are kept in a state of constant nitrogen limitation that curbs their growth (Falkowski *et al.* 1993).

Another example of this type of regulation is seen in the relationship between *Paramecium bursaria* and its symbiotic chlorellae. The host protozoan is believed to impose synchronization of division on its algal population to control its growth (Weis 1977). Certain insects may also display this type of indirect control. Numerous reports indicate that insects have limited physiological control over the location and the density of their symbionts (see Douglas 1989 for a review). Blood-sucking insects such as Tsetse flies require symbiotic microorganisms for metabolism. It appears that the insects are capable of partitioning and controlling their symbionts within a specialized structure called the mycetome (Nogge and Ritz 1982).

In direct population density control of partners, some partners are actually destroyed. For example, the bioluminescent squid described previously not only regulate luminescence of their symbionts they also expel all excess symbionts (produced through the previous night's geometric growth) at sunrise. It is hypothesized that this stops overgrowth of the light organ and decreases the metabolic cost to the squid (Boettcher *et al.* 1996). A second example of the direct density regulation of a mutualistic partner is seen in the yucca/yucca moth mutualism (Addicott 1986, Pellmyr and Huth 1994). This example will be discussed in detail in the next section.

Mutualisms possess interesting dynamics and complexity. The context dependency of many interactions leads to variable costs and benefits and thus conditionality. Many of the systems that display conditionality also show some evidence of partner regulation, which allows partners to control costs and benefits. An understanding of conditionality and subsequent regulation provides insight into how mutualisms remain stable and persist in the face of ever changing conditions, and in the face of the selfish interests of both partners.

1.0.6 Yuccas and Yucca Moths

A detailed study of conditionality and subsequent regulation and how this stabilizes an interaction will be examined in the context of the yucca/yucca moth mutualism. The uniqueness of the relationship between yuccas and yucca moths has been recognized since the late 1800's (Engelmann 1872, Riley 1872, 1892a) and it has been studied ever since (selected references: Powell and Mackie 1966, Aker and Udovic 1981, Keeley *et al.* 1984, Addicott 1986, James *et al.* 1993, Pellmyr and Huth 1994, Ziv and Bronstein 1996). Unlike many other pollination systems that are facultative and characterized by the participation of a number of non-specific pollen vectors, the female yucca moth is believed to be the only pollinator for the majority of yuccas (Riley 1892a, but see Dodd and Linhart 1994). This system is further complicated because the moth functions simultaneously as an obligate pollinator and obligate seed predator on the plant (Riley 1892a, Powell and Mackie 1966). Passive pollination of yucca flowers is an unlikely event because the pollen is sticky and glutinous, the anthers are usually well below the top of the style and the surface of the stigma is actually recessed within the style (Riley 1892b, but see Dodd and Linhart 1994). To accomplish pollination, mated female yucca moths must first collect the sticky pollen by placing the base of the head on the anthers and then using the front legs and the unique structures called the maxillary tentacles to form the adhering pollen into a tight ball (Riley 1892b). Males do not possess maxillary tentacles. After pollen collection, females normally fly to a new plant and most yuccas are generally self-incompatible (Aker and Udovic 1981, Fuller 1990, James *et al.* 1993, Richter 1995, Pellmyr *et al.* 1997). Preceding pollination, the female yucca moth will oviposit into the ovary of the flower. After one to several eggs are laid the female will approach the tip of the style uncurl the special maxillary tentacles and use them to force pollen grains into the opening and down to the stigmatic surface (Riley 1873). The ovary of the pollinated flower may then develop into a fruit that will contain the moth progeny. The developing larvae will then eat the developing seeds contained in the fruit of the plant. Only 5-15% of the total flowers per plant will be successfully matured to fruit with the plant abscising the rest (Aker 1982, Addicott 1986). Abscised flowers containing moth eggs represent a complete fitness loss to both the plant and the moth (Wilson and Addicott 1998).

The situation between yucca moths and yuccas has been described as a balanced conflict (Fuller 1990). Moth fitness increases with higher egg loads per flower (Pellmyr and Huth 1994), however, an increase in moth fitness represents a decrease in the number of uneaten seeds and thus yucca fitness. If only yucca fitness is considered it can be seen that the plant suffers under both high and low moth densities or visitation. Under low densities the plant is pollen limited and thus may not be able to maximize seed set. However, under high moth densities the plant fitness also decreases, as the number of seed predators is greater. This is because each flower may receive ovipositions from several moths. This mutualism clearly has outcomes that are conditional because costs and benefits to both moth and plant are highly variable. In terms of long term energy expenditure, the high moth visitation situation may be more critical to the yucca because more resources are spent on partially developing fruit whereas in the low density situation, flowers with insufficient pollination are dropped before any more resources are invested (Aker and Udovic 1981, personal observations). Thus some hypothetical optimal partner density or level of moth visitation may exist.

As in many conditional mutualisms, this relationship could clearly shift towards a parasitism with the moth taking complete advantage of the plant. However field observations of larval emergence and of seeds remaining uneaten within fruits indicate this rarely happens. Since it has been observed that moths lay enough eggs in flowers for the larvae to consume all seeds (Addicott 1986) but in the retained fruit this rarely occurs indicates that some sort of regulatory mechanism must exist. Bull and Rice (1991) hypothesized that in high moth visitation situations the plant could retaliate against the moth or regulate the mutualism by selectively abscising over utilized flowers. It was then experimentally demonstrated that indeed yuccas use selective abscission of surplus flowers to regulate costs relative to the benefits received from moths (Pellmyr and Huth 1994, Huth and Pellmyr 1997, but see Addicott and Bao in review). There is a strong negative relationship between moth egg number and the probability of flower retention (Pellmyr and Huth 1994) suggesting that selective abscission against high egg loads is one way that the plant regulates the moth.

The main purpose of the present study is to explore further aspects of partner regulation in this mutualism. In Chapter 2, I examine yucca fruit set patterns to determine if they have a regulatory function. Yuccas, like many other plants, have non-random fruit retention patterns (Aker and Udovic 1981) and I speculated that this might be relevant for partner regulation in this mutualism. I confirmed the non-random retention of yucca fruit and then measured several fruit and larval features in relation to this non-random pattern. I was interested in determining if fruit from different parts of the inflorescence have different fitness payoffs for the yucca host and how this might influence the balance of the conflict.

In Chapter 3, I explore what other factors besides density of moth ovipositions trigger regulation through selective abscission. In other plant species extrinsic factors such as pollen quantity and quality are known to affect flower fate (Stephenson *et al.* 1988, Becerra and Lloyd 1992) as well as intrinsic factors such as flower position on the inflorescence or flowering sequence (Sutherland 1987). I investigated how amount of pollen delivered to stigmas and flower position on the inflorescences affect flower fate first separately and then in conjunction with moth ovipositions.

The results from this thesis add to our understanding of conditionality and regulation in mutualism. The conflict caused by the selfish interests of both partners, and how this conflict is resolved for continued mutual benefit, provides insight into how mutualisms evolve and persist.

Species 2 ↓	*Species 1* **Negative (-)**	**Neutral (0)**	**Positive (+)**
Negative (-)	(-,-) competition	(-,0) amensalism	(-,+) predation/parasitism
Neutral (0)	(0,-)	(0,0) neutralism	(0,+) commensalism
Positive (+)	(+,-)	(+,0)	(+,+) **mutualism**

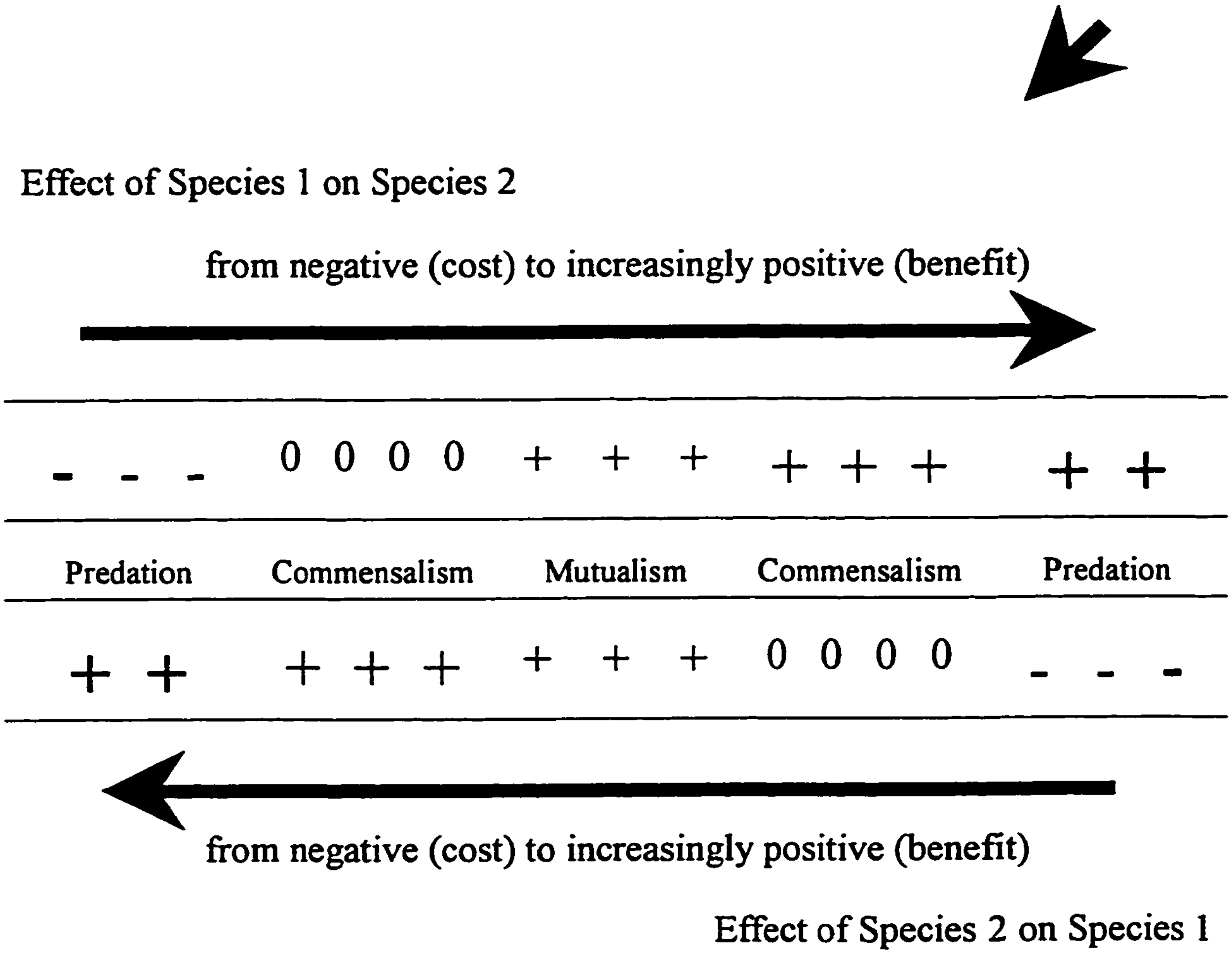


Figure 1.1. Redrawn and adapted from Bronstein (1994b). The upper part shows the classic interaction grid between two species. The lower panel shows how the mutualism box may be expanded to reflect the dynamic nature of interactions.

1.1 LITERATURE CITED

Abrams, P. A. 1987. On classifying interactions between populations. Oecologia **73**:272-281.

Addicott, J. F. 1986. Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. Oecologia **70**:486-494.

Addicott, J. F. 1995. The ecology of mutualism. In: The Encyclopedia of Environmental Biology. Academic Press pp. 601-611.

Addicott, J. F. and T. Bao. Limiting the cost of mutualism: multiple modes of interaction between yuccas and yucca moths. In review.

Aker, C. L. 1982. Regulation of flower, fruit, and seed production by a monocarpic perennial, *Yucca whipplei*. Journal of Ecology **70**:357-372.

Aker, C. L. and D. Udovic. 1981. Oviposition and pollination behavior of the yucca moth, *Tegeticula maculata* (Lepidoptera: Prodoxidae), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). Oecologia **49**:96-101.

Axelrod, R. and D.W. Hamilton. 1981. The evolution of cooperation. Science **211**:1390-1396.

Becerra, J. X. and D. G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormia tenax* (Agaraceae): a second action of self-incompatibility at the whole flower level? Evolution **46**:458-469.

Boettcher, K. J., E. G. Ruby and M. J. McFall-Ngai. 1996. Bioluminescence in the symbiotic squid *Euprymna scolopes* is controlled by a daily biological rhythm. Journal of Comparative Physiology. A, Sensory, Neural and Behavioral Physiology **179**:65-73.

Breton, L. M. 1990. Conditional mutualism: density- and quality-dependent responses of aphids to tending by ants. MSc Thesis. University of Alberta, Canada.

Bronstein, J. L. 1994a. Our current understanding of mutualism. The Quarterly Review of Biology **69**:31-51.

Bronstein, J. L. 1994b. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution **9**:214-217.

Brooks, W. R. and C. L. Gwaltney 1993. Protection of symbiotic cnidarians by their hermit crab hosts: evidence for mutualism. Symbiosis **15**:1-13.

Bull, J. J. and W. R. Rice. 1991. Distinguishing mechanisms for the evolution of co-operation. Journal of Theoretical Biology **149**:63-74.

Cushman, H. T. and J. F. Addicott. 1991. Conditional interactions in ant-plant-herbivore mutualism. In Culter, D. F.and C. Huxley (eds.) Ant-Plant Interactions. Oxford University Press.

Cushman, H. T. and T. G. Whitham. 1989. Conditional mutualism in a membracid-ant association: temporal, age-specific, and density-dependent effects. Ecology **70**:1040-1047.

Davidson, D. W. and S. R. Morton. 1981. Myrmecochory in some plants (*F. chenopodiaceae*) of the Australian arid zone. Oecologia **50**:357-366.

Dodd, R. J. and Y. Linhart. 1994. Reproductive consequences of interactions between *Yucca glauca* (Agavaceae) and *Tegeticula yuccasella* (Lepidoptera) in Colorado. American Journal of Botany **81**:815-825.

Donaldson, J. S. 1997. Is there a floral parasite mutualism in cycad pollination? The pollination biology of *Encephalartos villosus* (Zamiaceae). American Journal of Botany **84**:1398-1406.

Douglas, A. E. 1989. Mycetocyte symbiosis in insects. Biological Review **64**:409-434.

Engelmann, G. 1872. The flower of yucca and its fertilization. Bulletin of The Torrey Botanical Club **3**:33.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and L. McCloskey. 1993. Population control in symbiotic coral. BioScience. **43**:606-611.

Forester, A. J. 1979. The association between the sponge *Halichondrea panicea* (Pallas) and scallop *Chlamys varia* (L.): a commensal-protective mutualism. Journal of Experimental Marine Biology and Ecology **36**:1-10.

Frank, S. A. 1995. The origin of synergistic symbiosis. Journal of Theoretical Biology **176**:403-410.

Fuller, O. S. 1990. Factors affecting the balance of co-operation and conflict between the yucca moth, *Tegeticula yuccasella* and its mutualist, *Yucca glauca*. PhD Thesis, University of New Mexico, Albuquerque, New Mexico, USA.

Heil, M., B. Fiala, K.E. Linsenmair, G. Zotz, P. Menke, and U. Maschwitz. 1997. Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in anti-herbivore defence via symbiotic ant partners. Journal of Ecology **85**:847-861.

Huth, C. and O. Pellmyr. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. Oikos **78**:576-584.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1993. Pollination ecology of *Yucca elata*. An experimental study of a mutualistic association. Oecologia **93**:512-517.

Janzen, D. H. 1985. The natural history of mutualism. In Boucher, D. H. (ed.). The Biology of Mutualism. Oxford University Press. pp.40-99.

Johnson, N. C., J. H. Graham and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist **135**:575-586.

Keeler, K. H. 1985. Cost:benefit models of mutualism. In Boucher, D. H. (ed.). The Biology of Mutualism. Oxford University Press. pp.100-127.

Keeley, J. E, S. C. Keeley, C. C. Swift, and J. Lee. 1984. Seed predation due to the Yucca-moth symbiosis. American Midland Naturalist **112**:191-197.

Leimar, O. and A. H. Axen. 1993. Strategic behavior in an interspecific mutualism: interactions between lycaenid larvae and ants. Animal Behavior **46**:1177-1182.

Losey, G. S. 1979. Fish cleaning symbiosis: proximate causes of host behavior. Animal Behavior **27**:669-685.

McLean, R. B. and R. N. Mariscal. 1973. Protection of a hermit crab by its symbiotic sea anemone *Calliactis tricolor*. Experientia **29**:128-130.

Marcus, M. J. 1984. Feeding accociations between capybaras and jacanas: a case of intraspecific grooming and possibly mutualism. Ibis **127**:240-243.

Murray, K. G., S. Russel, C. M. Picone, K. Winnett-Murray, W. Sherwood, and M. L. Kuhlmann. 1994. Fruit laxatives and seed passage rates in frugivores: consequences for plant reproductive success. Ecology **75**:989-994.

Muscatine, L. and J. W. Porter. 1977. Reef corals: mutualistic symbioses adapted to nutrient poor environments. BioScience **27**:454-460.

Nogge, G. and R. Ritz. 1982. Number of symbionts and its regulation in Tsetse flies, *Glossina* spp. Entomolgia Experimentalis et Applicata **31**:249-254.

O'Dowd, D. J. 1979. Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. Oecologia **43**:233-248.

O'Dowd, D. J. and M. E. Hay. 1980. Mutualism between harvester ants and a desert ephemeral: seed escape from rodents. Ecology **61**:531-540.

Pellmyr, O. and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature **372**:257-260.

Pellmyr, O., L. K. Massey, J. L. Hamrick and M. A. Feist. 1997. Genetic consequences of specialization: yucca moth behavior and self-pollination in yuccas. Oecologia **109**:273-278.

Pierce, N. E., D. R. Nash, M. Baylis and E. R. Carpenter. 1991. Variation in the attractiveness of lycaenid butterfly larvae to ants. In Culter, D. F.and C. Huxley (eds.) Ant-Plant Interactions. Oxford University Press.

Powell, J. A. and R. A. Mackie. 1966. Biological interrelationships of moths and *Yucca whipplei* (Lepidoptera: Gelechiidae, Blastobasidae, Prodoxidae). University of California Publications in Entomology **42**:1-59

Richter, K. S. 1995. The pollination of *Yucca whipplei* by *Tegeticula maculata*: the benefits and costs of an obligate mutualism. Ph.D. Dissertation. University of California, Irvine. USA.

Riley, C.V. 1872. The fertilization of the yucca plant by *Pronuba yuccasella*. Canadian Entomologist **4**:182.

Riley, C. V. 1873. On the oviposition of the yucca moth. American Naturalist 7:619-623.

Riley, C. V. 1892a. The yucca moth and yucca pollination. Annual Report of the Missouri Botanic Garden **3**:99-159.

Riley, C. V. 1892b. Some interrelations of plants and insects. Insect Life **4**:358-378.

Sazima, M. and I. Sazima. 1978. Bat pollination of the passion flower, *Passiflora mucronata*, in southeastern Brazil. Biotropica **10**:100-109.

Schemske, D. W. 1980. Floral ecology and hummingbird pollination of *Combretum farinosum* in Costa Rica. Biotropica **12**:169-181.

Schiestl, F., M. Ayasse, H. F. Paulus, D. Erdmann and W. Francke. 1997. Variation of floral scent emission and postpollination changes in individual flowers or *Ophrys sphegodes* subsp. *sphegodes*. Journal of Chemical Ecology **23**:2881-2895.

Setälä, H., J. Rissanen and A. M. Markkola. 1997. Conditional outcomes in the relationship between pine and ectomycorrhizal fungi in relation to biotic and abiotic environments. Oikos **80**:112-122

Stephenson, A. G., B. Devlin, and J. B. Horton. 1988. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Curcurbita pepo* (Zucchini squash). Annals of Botany **62**:653-661.

Stout, J. 1979. An association of an ant, a mealy bug, and an understory tree from a Costa Rican rainforest. Biotropica **11**:309-311.

Sutherland, S. 1987. Why hermaphroditic plants produce many more flowers than fruits: experimental tests with *Agave mckelveyana*. Evolution **41**:750-759.

Tomback, D. F. 1982. Dispersal of whitebark pine seeds by Clark's nutcracker: a mutualism hypothesis. Journal of Animal Ecology **51**:451-467.

Weis, D. 1977. Synchronous development of symbiotic chlorellae within *Paramecium bursaris*. American Micropial Society Transactions **96**:82-86.

Wilson R. D. and J.F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos **81**:109-118.

Ziv, Y. and J. L. Bronstein. 1996. Infertile seeds of *Yucca schottii*: a beneficial role for the plant in the yucca-yucca moth mutualism? Evolutionary Ecology **10**:63-76.

Chapter 2

Regulation of the Mutualism Between Yuccas and Yucca Moths: Patterns and Consequences of Fruit Set

2.0 INTRODUCTION

Non-random fruit set patterns are well documented in plants with surplus flowering strategies. These intrinsic patterns have been linked to flower position on the inflorescence or flower timing in the blooming sequence (Wyatt 1982, Holtsford 1985, Sutherland 1987, Stephensen *et al.* 1988, Guitian 1994, Diggle 1995) as well as to non-random pollinator visitation patterns (Berry and Calvo 1991, Goldingay and Whelan 1993). These non-random patterns of fruit set can also translate into differences in the characteristics of the fruit themselves. Differences in fruit characteristics tied to differences in the position on the inflorescence that the fruit occupies can include fruit size, seed number and seed viability (Lovett Doust *et al.* 1986, Herrera 1991, Winn 1991, Obeso 1993a).

Yuccas (Agavaceae) are usually resource-limited and mature less than 20% of their flowers into fruit (*Yucca whipplei,* Aker 1982; *Y. elata,* James *et al.* 1993; *Y. glauca,* Dodd and Linhart 1994; *Y. filamentosa,* Pellmyr and Huth 1994; *Y. kanabensis,* Addicott and Tyre 1995). In addition, the locations of the few successful flowers are not random (Aker 1982, Huth and Pellmyr 1997). These non-random patterns of fruit set are linked to differences in overall fruit quality. In *Y. whipplei,* seeds from fruit occurring at the bottom of the inflorescence are heavier than seeds from top fruit (Aker 1982) and *Y. whipplei* preferentially retain bottom fruit over those from the top (Aker and Udovic 1981). However, it is unknown whether non-random patterns of fruit retention are caused by some aspect of extrinsic pollinator visitation behavior or if intrinsic inflorescence architecture is driving the patterns seen in yuccas.

High rates of abscission and non-random patterns of fruit set are not unique to yuccas. However, details of the yucca pollination system make these factors particularly interesting. First, yuccas have an active, efficient and coevolved pollination mutualism with yucca moths (Riley 1872, reviewed in Baker 1986). Second, moths are also seed predators, laying eggs in the flowers prior to pollinating them. Third, high rates of flower abscission and non-random patterns of fruit retention have direct consequences for pollinators because abscised flowers and immature fruit represent a complete fitness loss to moths that visited that flower (Wilson and Addicott 1998). Fourth, some yuccas use selective abscission to protect fitness interests (Pellmyr and Huth 1995, Richter 1995, Addicott 1998). In addition, intrinsic plant features such as the presence of inviable seeds (Ziv and Bronstein 1996) and the hardening of the seed coat might protect the plant by deterring larval feeding.

The goal of the present study was to document quantitatively the existence of intrinsic non-random patterns of fruit retention in *Yucca kanabensis* McKelvey and to determine if these non-random patterns affect yucca fitness. For example, if intrinsic non-random fruit retention patterns result in fruit of higher quality, these fruit will improve plant fitness. This might occur, for example, if the retained fruit have more numerous or larger seeds. These intrinsic patterns of fruit set favouring fruit of higher quality might also incidentally decrease costs incurred by feeding yucca moth larvae. For example, if high quality fruit contain more seeds, then the plant may lose a lower proportion of seeds to larval feeding. Hence, if non-random fruit retention patterns are caused by differences in fruit quality these patterns may provide an intrinsic regulatory mechanism to this mutualism.

The present study had several objectives. First, I wanted to determine what the intrinsic fruit set patterns (if any) were for *Y. kanabensis*. This was done experimentally by hand pollinating flowers and excluding moths. The flowers on these plants received controlled but excess pollen and no moth ovipositions. These flowers represented the highest quality possible and I considered this to be the intrinsic pattern of fruit set. The second objective was to investigate if the fruit set patterns seen are adaptive to the plant by decreasing the costs incurred from the moth larvae. I hypothesized this might occur in two different ways.

First, fruit set patterns might directly influence fruit qualities such as seed size, number or development rate. This could in turn influence the costs yuccas incur from the feeding moth larvae. For example, a larger fruit could accommodate more larval feeding than a smaller fruit.

The second way that fruit retention patterns might contribute to regulation of this mutualism is if these patterns can influence larval qualities. For example, if fruit from different areas of the inflorescence mature at different rates or are different sizes this might in turn affect the quality or availability of food resources for larvae. I speculated that these differences might manifest themselves in differences in larval weights, larval consumption patterns or emergence dates.

2.1 METHODS

2.1.1 Study organisms and study site

I studied populations of *Yucca kanabensis* McKelvey located west of Kanab, Kane County, Utah, USA. The vegetation on the sandy ridges located in this area is composed of *Yucca kanabensis* McKelvey, *Quercus gambelli* Nutt., *Juniperus monosperma* Engelm., *Artemesia tridentata* Nutt., and *Wyethia scabra* Nutt.. Addicott and Tyre (1995) provide detailed descriptions of study sites and study organisms. Experiments were carried out during the flowering seasons of 1995 and 1996. I performed the ALL FLOWERS POLLINATED experiment during 1995 at the site located on the road to Coral Pink Sand Dunes State Park (112° 40' 45" W, 37° 7' 30" N, 1800 m elevation) near Yellowjacket Ranch. The other two experiments, FRUIT DIFFERENCES and MOTH DIFFERENCES were performed on the north side of US Highway 89 on the sandy ridge between Kanab and Mt. Carmel Junction (112° 36' 30" W, 37° 10' 15" N 1838 m elevation) in 1995 and 1996 respectively.

Yucca kanabensis is a narrow leafed, xerophytic monocot in the Agavaceae. It reproduces both vegetatively and from seed. Vegetative growth of this long-lived polycarp is characterized by formation of clumps or clones of leafy rosettes. Individual rosettes do not flower every year. The taxonomic status of the yucca populations described at the location above is unresolved (McKelvey 1947, Webber 1953, Cronquist *et al.* 1977) and I prefer to follow the classification of McKelvey (1947) until the taxonomic status is revised using molecular characters.

Several studies examining flowering and fruiting patterns have been done on yuccas that are branched (*Y. whipplei*, Aker and Udovic 1981, Richter 1995; *Y. elata*, James *et al.* 1994; *Y. filamentosa*, Huth and Pellmyr 1997). The present study uses *Y. kanabensis*, which has an unbranched inflorescence (a raceme), making it relatively easy to study flowering and fruiting patterns. Elongating inflorescences first appear in May or June. Flowering is acropetal, beginning with the flower buds most proximal to the leaves of the basal rosette and proceeding upwards. Although the inflorescence is unbranched, more than one flower bud may occur at every flower bearing node or axil. Multiple flowers at the same axil position are not at the same developmental stage. There is approximately a three-day difference in development between these flowers. The first flower to open at each axil is designated as the wave 1 flower, the second to open, wave 2 and so on. Although flowering within a wave is acropetal, the different flowering waves overlap, so a few nights after the wave 1 flowering has started and proceeded part way up the inflorescence, the wave 2 flowers begin opening at the bottom of the inflorescence. Thus it is common to have fresh wave 1 flowers located near the top of the inflorescence and fresh wave 2 flowers located below it. Two waves of flowers are most common but three or more waves can occur on some very robust plants.

Yucca flowers are hermaphroditic and usually begin opening in the early evening with full anthesis by dusk. They remain attractive to moths and receptive to pollen for two nights. Flowers then begin to wilt but remain attached to the inflorescence for about a week, and either abscise or begin to show signs of ovary enlargement. *Yucca kanabensis* fruit development is similar to that of other yuccas (*Y. whipplei*, Powell and Mackie 1966; *Y. schottii*, Powell 1984) with fruit reaching full size in less than 30 days and final seed maturation taking about another 30 days. Eventually the fruit dries and splits, slowly releasing the dry, black, lightweight, wind-dispersed seeds.

Flowers are pollinated by small, white, nocturnally active yucca moths, *Tegeticula yuccasella* Riley. These moths are members of an unresolved species complex (Addicott and Tyre 1995, Addicott 1996, Pellmyr *et al.* 1996). There are three undescribed, but distinct, members of this complex on my sites. These three species of moth are designated as deeps, shallows and secondaries, based upon differences in where and when the female moths lay eggs. The deep and shallow female moths have specialized mouthparts that allow them to actively transfer pollen. I will refer to these as pollinators. The secondary females do not have these structures, and are thus non-pollinating seed predators. Female pollinators lay eggs in the pistils of the flowers they pollinate. The moth larvae hatch and develop as seed predators within the developing yucca fruit. Larval development of the species on my sites can be completed in 30 days (similar to Riley 1892, Rau 1945) at which time the larvae chew out of the fruit, fall to the sand, burrow in, and cocoon. Pupation is then completed in a subsequent spring. Yuccas and yucca moths are completely reliant upon each other for successful sexual reproduction.

2.1.2 Hand Pollination Protocol

All three experiments presented in this chapter rely on the following procedure for hand pollination of flowers. I chose pollen donor plants located at least 500 m from any recipient plants. Initially I removed any open flowers and then enclosed each donor plant in a long tubular, zippered bag made of flexible fibre glass window screening and fine netting to exclude moths. On each subsequent morning I removed all fresh flowers and placed them in a cooler until required for pollen transfers. I randomly chose flowers from the cooler, removed the petals, and carefully detached the crude pollinia from each anther with a yucca leaf blade, transferring the mass of pollen to one of the removed petals. Pollen loads were then carefully transferred to the recipient flower and gently placed down the style. No effort was made to ensure that each flower on a recipient plant received pollen from the same or different donors. All pollinations were complete within 6 hours and there was no concern that pollen viability had decreased over this time. This was because an experiment done with pollen aged between 1 and 11 days showed that there is no difference in flower retention between pollen that is fresh and up to 6 days old.

In fact 11 day old pollen was observed to result in a few fruit (S. Humphries unpublished data).

Experiments used different pollen loads ranging from low to excessive. The low level was equivalent to a quarter of the number of pollen grains contained on one anther, the high level was equivalent to the amount contained on one anther, and the excess level was the amount contained on three anthers. Hence pollinia were either combined for the excess level equivalent to 3 standard anther loads (SAL), left intact for 1 SAL or divided into roughly equal quarters for the ¼ SAL treatment. The high pollen level is similar to the heaviest pollen levels naturally pollinated flowers receive (JFA unpublished data). All three pollen levels have enough grains to fertilize all of the ovules in a *Y. kanabensis* flower, as each anther has ca. 4000 pollen grains (mean=4145, sd=407.5 n=6 flowers) and each flower has an average of 318 ovules (range 209-432, sd=51.8, n=70). Pollen was mechanically counted using a Coulter Counter ® following the methods of Harder *et al.* (1985).

2.1.3 Experiments

Three major experiments were carried out during the flowering seasons of 1995 and 1996. The first experiment, ALL FLOWERS POLLINATED, was intended to determine the intrinsic fruit retention pattern of *Y. kanabensis*. Previous observations of extrinsic fruit set patterns under natural pollination from these study populations indicated that fruit retention was not random (JFA unpublished data) and that plants had preferred and non-preferred regions for setting fruit. The second and third experiments were then designed to examine fruit and larvae from the yucca's naturally preferred and non-preferred regions of fruit set. I speculated that fruit retention patterns might result in differences in the overall qualities of the fruit themselves (FRUIT DIFFERENCES) or the larvae within (MOTH DIFFERENCES).

2.1.3.1 All Flowers Pollinated – Intrinsic Fruit Retention Patterns Without Moths

The first experiment had two objectives. The first was to determine the intrinsic pattern of fruit set in the absence of variation in moth ovipositions and pollination. That is, given adequate pollination of all flowers, which flowers become mature fruit? The second objective was to determine if this intrinsic pattern of fruit set is altered when all flowers receive high amounts of pollen (1 standard anther load=1 SAL) or when all flowers receive just an adequate amount of pollen (quarter of a standard anther load=¼ SAL).

At the Yellowjacket Ranch site I haphazardly selected fifteen clones each bearing two or more inflorescences. Clones with at least two flowering stalks were required for a blocked design to account for differences in fruit retention within and between clones. I then randomly assigned two inflorescences from each of these clones to either the high or low pollen treatment (n=30 inflorescences). These plants were bagged to protect them from cows, deer and moths.

All the flowers on one inflorescence in each clone received just ¼ SAL of pollen, while the flowers on the second inflorescence received 1 SAL of pollen. I inspected plants daily from when flowering began on June 13 until it ended on July 8. I identified all flowers by their axil position and flowering wave by writing on the outside of the sepals with a Sharpie® Permanent Marker as the flowers opened. Beginning at sunrise I visited the plants, inspecting and pollinating the flowers that had opened the previous night and checking the status of previously opened flowers daily until the plant had finished flowering. After flowering ended plants were again checked daily until the youngest remaining flower on the plant was 3 weeks old. When abscission was complete, the origin and fate of each flower was known and this information was used to examine the patterns of fruit retention with controlled pollen levels but without moth ovipositions.

Due to differences in the flower displays between plants I tried to develop a system of ordering or ranking flowers so that comparisons could be made across plants. Several methods were used and are considered below. Once the ranking was established, I then divided the flowers by rank into thirds and then within each third considered the proportion of flowers that became fruit. I chose to divide flowers into groups based on thirds because I required a simple way to assign flowers to groups for the manipulative experiments that followed from the first experiment. Thirds corresponds well with the bottom, middle and top of inflorescences.

2.1.3.2 Fruit differences

To investigate if the population level fruit retention patterns were related to flower quality (e.g. fruit size and seed number), I manipulated plants to set fruit in each region (bottom, middle and top) by only hand pollinating those flowers in the region of interest. I then allowed the fruit to mature, harvested them and measured various fruit characteristics including number of seeds, fruit length, fruit width, fruit and seed wet weight and seed dry weight.

At the US 89 site I haphazardly chose yucca clones with three or more inflorescences. I then randomly chose three inflorescences from within each clone and bagged them in fine mesh zippered cages to exclude pollinators and deer. Fruit position treatments were then assigned randomly to each of the three inflorescences in the clone. The number of axils for each inflorescence was determined and an estimate was made of the location of the upper, middle, and lower part of the inflorescence. Twelve wave 1 buds were left on the plant within the region that was specified. Fruit were induced to set by removing all of the buds except for those in the area of interest. As flowers opened, they were hand pollinated with 3 SAL of pollen, an amount known to be in excess of what is required to effect full pollination of ovules. Styles were also cut after 3 days to determine, by dissection, if pollen had germinated and if a consistent amount was being delivered each time. Fruit were left to mature for 30 days, which is before they were fully mature (~ 60 days), but after they had reached full size. All fruit were harvested at the same age. Harvested fruit were then stored at 4°C until dissected. Fruit were split along their carpel boundaries and the seeds were removed from each of the six locules. The seeds were weighed to establish seed wet weight and were then stored in 70% ethanol until drying was possible. Seeds were dried to a constant weight at 50°C, then weighed and counted. Because seeds were taken from fruit that were not ripe, viability tests through germination were not attempted.

2.1.3.3 Larval Differences

The last experiment was designed to determine if fruit differences between the top, middle and bottom of the inflorescence resulted in larval differences. I tested whether fruit from different areas of the inflorescence recruit more larvae and if larval feeding damage is the same.

Clone selection and treatment assignment procedures were identical to those described above for the FRUIT DIFFERENCES experiment. The three possible treatments were fruit at the bottom, middle and top. I removed all buds outside the area that was the assigned plant treatment, but unlike the previous experiment I left the remaining flowers open to natural moth pollination and oviposition. I made no effort to control the number of moths visiting experimental flowers.

I inspected plants daily, checking for oviposition marks and evidence of moth pollination. If one-or two-day-old flowers had oviposition marks, I added 1 SAL of supplemental pollen to improve the chances of flower retention. Since flower opening date was known, the date that the moth eggs had been laid was also known (+/- 1 day). Fruit were then left to mature.

I began inspecting fruit daily for larval emergence after 30 days. I captured emerging larvae by placing plastic bags over individual fruit starting at 7:00 p.m. and removed the bags at 7:00 a.m. I speculated that larval emergence would most likely occur during this time because larvae are known to be susceptible to heat and light (Powell and Mackie 1966) and the major observed larval predators (ants and lizards) are less active. After a few days I had to modify this (due to some fruit breakage) and left the bags on throughout the day and night, instead inspecting the bags two or three times a day and removing any larvae. Plastic bags over the fruit did not seem to have an effect on larval emergence as bagged fruit had synchronous emergence with unbagged fruit as well as those bagged in fine mesh bags (personal observation). I chose plastic bags over mesh, in spite of humidity building up within the bags, because larvae were capable of chewing out of mesh bags.

Emerging larvae were taken back to the lab where they were weighed and measured (head capsule length and width). Emergence holes were also marked on the outside of fruit to help determine if larvae had emerged without being captured. A fresh unmarked emergence hole without a corresponding larva indicated an escape and new larvae without new emergence holes indicated several larvae sharing the same exit.

I harvested fruit at 45 days placing them in the fridge for an additional 2 days to allow for any final larval emergence before dissection. The picking of fruit often triggers final emergence of larvae that have already excavated tunnels to the surface and cool fridge temperatures do not larvae from leaving the fruit (and exploring the fridge!). I chose to harvest fruit at 45 days for two reasons. First, previous work indicated that larval development could be completed within 30-45 days (Riley 1892, Rau 1945). Second, since fruit were not bagged until 30 days, they were subject to attack from secondaries, the non-pollinating moths. These moths are 50% larger than the pollinators and their larvae inflict greater feeding damage (James 1998). Since these moths were not the focus of my investigation, I could only account for their damage if I removed fruit while the non-pollinators were still in the first or second instar of development. Both pollinators and non-pollinators have four instars.

During dissection, I determined the number of viable (black) and unviable (white) seeds as well as the number of seeds lost to larval feeding in each locule. If possible I also tried to determine how many larvae had been feeding in each locule (often ambiguous, as larvae will cross to adjacent locules). If larvae remaining in the fruit were encountered, I removed them, noted that they had not emerged naturally, and measured them in the same manner as those that had already emerged. Secondary larvae were easily recognized because they had only reached 2nd instar (and still occupied the original seed the egg was laid in) while both species of pollinators were in their 4th instar.

2.1.4 Statistical Analysis

I performed my data analysis using SPSS for Windows, version 7.5 using Goodness of Fit, Contingency, Logistic Regression, ANOVA Kruskal Wallis, and Mann-Whitney test procedures. Freidman's Test was calculated by hand as in Zar (1996).

2.2 RESULTS

2.2.1. All Flowers Pollinated

2.2.1.1 Phenology

Flowering in experimental plants began on June 13, 1995 and ended on July 8, 1995. Peak flowering occurred on June 26 with 231 fresh flowers from 30 inflorescences. Length of flowering for inflorescences ranged from 10–19 consecutive days. The average number of days until a pollinated flower abscised was 7.33 (n=2164 flowers) days (Figure 2.1). This indicates that natural flower abscission occurs at the time that pollinator larvae would be hatching suggesting that flower abscission decisions are not made in response to larval feeding.

2.2.1.2 Flower retention by wave

Table 2.1 shows the number of flowers, fruit and overall retention for both the low and high pollen treatments by flowering wave. Overall flower retention rate for both experiments was ~14%, which is similar to values reported for 1990 (13.3%) by Addicott (1998). There was no overall difference in the retention rate from the ¼ and 1 SAL treatments (Contingency $\chi^2=4.483$, df=2, p=0.106). Retention rates varied between waves of flowers. Retention rates were higher for wave 1 flowers followed by wave 2 and then wave 3 flowers (Table 2.1). Flower wave alone is a significant predictor of flower success (retention vs. abscission) with decreasing probability of retention as flower wave increases for both the 1 and ¼ SAL treatments (Table 2.2).

2.2.1.3 Flower retention by axil

Flower axil position, flower wave and flower opening date all affect flower fate, but these three factors are interrelated. To assess the importance of flower axil position alone (axil rank), I scored each flower as belonging to either bottom, middle or top of its inflorescence. This results in uneven sample sizes of flowers between the three sections because wave 2 and 3 flowers are more common in the middle 1/3 than either the top or bottom third. Considering axil rank alone, flowers from the bottom two thirds of the inflorescence were almost twice as likely to become fruit as those from the top third (Table 2.3) and there was no difference between the distribution of fruits between the 1 SAL and ¼ SAL plants (Contingency χ^2=2.270, df=2, p=0.321). Flower axil is also a significant predictor of flower success with decreasing probability of flower retention with increasing flower axil position (Table 2.4). A similar result is seen for the plants receiving the ¼ SAL pollen treatment (Table 2.4).

2.2.1.4 Flower retention by opening date (and axil)

For my second ranking, I ranked flowers by their opening date, and within each date I gave flowers on the lower axils lower ranks. I tried to incorporate these flowering features into my second flower ranking method. This made the temporal component of flowering the most important factor but within each date the spatial configuration of the flowers was incorporated. I then scaled the ranks from 0-1 for each inflorescence to allow comparisons for inflorescences of different sizes and grouped the ranked flowers into thirds. Unlike the previous ranking method, thirds now no longer correspond exactly with the bottom, middle and top of the yucca inflorescence.

Relative flower rank is a strong predictor of flower fate with flowers from the first third being almost twice as likely as the middle third of flowers and four times as likely as the last third of flowers to become fruit (Table 2.3, Table 2.5, Figure 2.2a). This retention pattern does not significantly differ between the 1 SAL and ¼ SAL treatments (Contingency χ^2=3.583, df=2, p=0.166).

2.2.2 Intrinsic flower/fruit differences

2.2.2.1 Flower differences

Three main factors could explain why not all groups of flowers are contributing equally to fruit set: 1) flower quality varies with wave, 2) flower quality varies with axil or 3) early flowers inhibit later flowers. To address whether flower quality varies with wave, I collected and dried the wave 1, 2 and 3 flowers from the first axil position that would bare all three flower waves on 30 randomly chosen plants. I then compared the mean flower dry weights of the three wave groups and found significant differences. Wave 1 flowers are heaviest followed by wave 2 flowers with wave 3 flowers being the lightest (Figure 2.3).

Second, to assess whether flower quality varies with axil I collected wave 1, 2 and 3 flowers from ~ 19 consecutive axil position on the last 2 flowering plants from 1997. Flowers were collected the day they opened and I measured flower wet weight. Figure 2.4 shows the flower weights plotted against axil position and the trend is surprisingly clear. Flowers from different waves have different weights and within each wave as you move up the inflorescence to higher axil positions flower weight also decreases. These trends are significant for both wave 1 and 2 flowers in spite of the small number sampled (Figure 2.4).

In addition to investigating if there were differences in flowers I also tested whether the patterns of fruit set might be related to differential quality of fruit from the different axil positions of the inflorescence. I speculated that fruits of different quality might alter the costs incurred by moth feeding. For example if some fruits were larger I would expect that these fruits would lose less seeds to larval feeding. To determine if fruit set patterns allow yuccas to decrease costs incurred by larval feeding two experiments were done. The first attempted to determine if there were actual differences in fruit qualities from different axils on the inflorescence and the second to see if there were differences in larval qualities from the different parts of the inflorescence.

2.2.2.2 Fruit differences

Fruit were forced to set in the preferred and non-preferred regions of the yucca inflorescence by only pollinating wave 1 flowers in the bottom, middle or top third of the flowering stalk. Moths were excluded from these fruit and after a 30-day maturation period I harvested 70 fruit from 15 inflorescences. Inflorescences within a clone tended to maintain the same number of fruit but the interclone variation was large (Range 3-7 fruit). I weighed and measured whole fruit and then dissected them to examine the seeds.

One unexpected result was the difference in seed developmental stage between fruit harvested at the same age (30 days after hand pollination). I examined and eliminated weather variation during the maturation period because all fruit developed within almost the same 30 day (+/- 5 days) period and the daily temperatures were virtually the same through the entire period. At 30 days some fruit were fully ripe with dehiscence beginning and seeds completely black and dry. In contrast some fruit were completely unripe with seeds still white and moist. A subjective classification of maturity was developed with 4 categories: (1) all seeds white and moist; (2) less than half of the seeds turning black; (3) at least half of the seeds were turning black; (4) all viable seeds black and dry. Fruit maturity was analyzed using the non-parametric Freidman's test because the observations were ranked. There were no significant differences in maturity between the three positions (Freidman's Test χ^2=2.7, df=3 treatments, 5 blocks, p>0.05).

I compared the other fruit features that I measured (seed wet weight, seed dry weight and seed total) using Analysis of Variance, blocking by clone. Multiple observations within each inflorescence, within each treatment were averaged to account for psuedoreplication. All fruit characters that I measured showed high variability and no significant differences in means between the three fruit positions (Table 2.6). Data for average seed wet weight per seed is presented (Figure 2.5) as an example. My inability to measure any differences between the fruit may be due in part to unexpectedly high variability within treatments and small sample sizes. The power to detect differences was low, ranging from 14%-22% (Table 2.6).

2.2.3 Larval Differences

Overall there were 90 harvested fruit, 20 from the bottom treatment, 35 middle treatment and 35 top treatment. A total of 927 larvae (837 pollinators and 90 secondaries) were found in the fruit. Over half of the pollinators emerged naturally between 30 and 45 days. The rest of the larvae were recovered by dissection. Twelve larvae escaped either while bags were off the fruit or while the fruit were in the fridge. The remaining 915 larvae were classified as secondaries, emerged pollinators, dead pollinators and non-emerged pollinators and the relative percentages of these larvae for each fruit position are summarized in Figure 2.6. The number of pollinator larvae per fruit did not differ between fruit from the bottom, middle or top of inflorescences (Kruskal Wallis, df=2, p=0.08, Figure 2.7a.).

2.2.3.1 Seeds eaten per larva

The average number of seeds eaten per pollinator larva was between 10 and 15 (Figure 2.7b) which is consistent with other studies (Powell and Mackie 1966, Addicott 1986). The average number of seeds eaten per larva differs among position treatments with larvae in the fruit from the top treatment eating significantly fewer seeds than those larvae in middle and bottom fruit (Table 2.7).

2.2.3.2 Larval Emergence

Most larval emergence occurred between 7:00 p.m. and 8:30 a.m., but some emergences occurred as late as 10:30 a.m. on cloudy, cool days. I also observed a relationship between weather and the likelihood of larval emergence as predicted by Powell and Mackie (1966). Larval emergence seemed to coincide with cool overcast rainy mornings and a dip in the mean temperature (Figure 2.8). Overall 81% of emergences occurred on such rainy days.

2.2.3.3 Emergence by Treatment

The percentage of larvae emerging by 45 days differed among treatments. Most pollinators emerged from the bottom fruit with approximately 25% more than the group with the least emergences, which were the top fruit (Figure 2.9a). Of the emerging larvae the mean age at emergence for bottom fruit and middle fruit was the same (37.6 days, Figure 2.9b) and this was significantly greater than the age of the larvae emerging from the top fruit, (Kruskal Wallis $\chi^2=155.95$, df=2, p<0.001).

2.2.3.4 Weight and Head Capsule Width

There were no significant differences in larval body weight or head capsule width between the three fruit position treatments for those larvae that emerged (weight: Kruskal Wallis df=2, p=0.838; head capsule: width-Kruskal Wallis df=2, p=0.157). However, there are significant differences in weights of larvae that emerged naturally or were removed from fruit after 45 days by dissection (Mann-Whitney U=63998.0, n=835, p<0.001). Larvae that emerge are heavier than those that were removed by dissection (emerged naturally: mean=0.071 g, sd=0.017, n=492; dissected: mean=0.062 g sd=0.040, n=343). But, there are no significant differences in larval head capsule width for emerging (mean=1.40 mm, sd=0.19 mm) and dissected larvae (mean=1.37 mm, sd=0.25 mm) (Mann-Whitney U = 83867.5, n=835, p=0.88) which is because they are all in the same instar. Head capsule sizes are consistent with *T. yuccasella* from other localities (Powell 1984).

2.2.3.5 Mortality of Pollinators

During dissection 68 fourth instar larvae that were recently deceased (based on color and level of decay) were recovered from the fruit. Bottom fruit had 5.4% mortality, middle fruit had 14.1% and top fruit had 3.7% (Figure 2.6). This does not appear to be a straightforward density effect as the middle and top fruit had similarly high densities of larvae but over a 3-fold difference in mortality.

2.3 DISCUSSION

Fruit retention patterns have been described for other yuccas and their close relatives (Aker and Udovic 1981, Aker 1982, Powell and Mackie 1966, Sutherland 1987, James *et al.* 1994, Huth and Pellmyr 1997). The results of these studies are highly variable (Table 2.8). My study is an important addition to those done previously because I used hand pollination of entire plants to determine the intrinsic pattern of fruit retention in an unbranched yucca. The non-random intrinsic retention patterns combined with data on flower differences within waves and among axils, adds to our understanding of the plant's underlying flower retention rules in the absence of variable pollen deposition and moth ovipositions.

2.3.1 The intrinsic pattern of Yucca kanabensis fruit retention

The intrinsic pattern of fruit retention in *Y. kanabensis* favors early flowers, those that are in the first wave and are located proximally in the inflorescence. This is similar to patterns recorded in other genera (Stephenson 1981, Bawa and Webb 1984, Wolfe 1992, Obeso 1993b). Three main factors could explain why not all groups of flowers are contributing equally to fruit set: 1) flower quality varies with wave 2) flower quality varies with axil or 3) early flowers inhibit later flowers.

Flowers compete for maternal resources (Stephenson 1981, Beccera and Lloyd 1992) and variation in competitive ability may be derived from spatial or temporal differences in timing between flowers or inherent differences in reproductive potential between flowers (reviewed in Diggle 1995). The first flowers to open in a sequence often have an advantage because of direct pre-emption of resources. Wave 1 flowers have this temporal advantage because they always open before the other waves at any given axil position.

The effects of inherent reproductive potential are more subtle and often involve differences in the number of ovules or the size of the ovules which likely translates into differences in sink strength. However, wave 1 flowers may be at an advantage, because they are heavier than the other flower waves at any given axil position (Figure 2.3 and Figure 2.4). This additional weight may influence flower retention by affecting the amount of resources a flower can draw (its sink strength) or it may represent the amount of energy the maternal plant has already invested in each flower.

Another explanation for the uneven contribution of flowers along the inflorescence to fruit set is that flower quality may vary by axil. Yucca flowers from lower axil positions tend to have a greater contribution to fruit set, which is well documented in other plants (Stephenson 1981, Bawa and Webb 1984, Wolfe 1992, Obeso 1993b). Again, this might be a function of flower timing in the sequence or inherent differences in flower reproductive potential that are spatially related. Since *Y. kanabensis* flowers acropetally, flowers at proximal axil positions open first. Again these flowers can pre-empt resources that would be available to later flowers. In addition there may also be differences in reproductive potential from flowers at different axil positions along the inflorescence (Lovett Doust *et al.* 1986, Winn 1991). By measuring flower weights of wave 1, 2 and 3 flowers at consecutive axil positions I was able to document a decline in flower weight as axil position increased. The pattern was consistent within all three waves of flowers.

2.3.2 Do fruit retention patterns affect fruit features?

The second objective of the present study was to determine if the patterns in fruit retention allow yuccas to regulate their interaction with yucca moths by allowing them to decrease their cost in the association. Since yuccas exchange seeds for pollination service, I expected an adaptive consequence of fruit set patterns might be measured as an increase in intact yucca seeds. When I manipulated the yucca plants to set fruit in the naturally preferred and non-preferred regions of the inflorescence I did not see any significant effects in the fruit qualities I measured. There were no significant differences in maturity rate, wet or dry seed weight or the total numbers of seeds per fruit. This is contrary to the study done by Aker (1982) who found that fruit from the bottom branches of *Y. whipplei* had heavier seeds than top fruit. I did not detect similar differences however my power to detect them was quite low. In addition, my fruit position treatments utilized only wave 1 flowers. Although this allowed me to investigate the differences in flowers from different axil positions along the inflorescence it ignored the obvious fact that yuccas preferentially retain wave 1 flowers and actively discriminate against their wave 2 and 3 flowers. An examination of fruit characters from wave 2 and 3 flowers would be useful.

2.3.3 Do fruit retention patterns affect larval features?

The final objective was to examine whether differences in fruit from the preferred and non-preferred regions of the yucca inflorescence might translate into larval differences. I speculated that if fruit from the bottom and middle of yucca inflorescences were larger they might lose fewer seeds to larval feeding. I did not detect any clear effect of fruit position on larval density, size or development rate. There was also no difference in the number of surviving seeds between the three treatments. Finally, I examined the consumption of seeds by individual larva and found that larvae in the top fruit (the non-preferred region) ate fewer seeds, which is the opposite of what I would predict if patterns of fruit set had adaptive regulatory consequences.

2.3.4 The extrinsic pattern of Yucca kanabensis fruit retention

Interestingly, there were differences in patterns of retention of flowers from hand pollinated plants (Figure 2.2a) and from plants that received pollen from yucca moths (Figure 2.2b). Using data collected from 1990 I examined extrinsic patterns of fruit set. Under a moth pollination regime flowers from the first rank group no longer have the highest chance of becoming fruit, but rather flowers from the middle rank group have the highest chance (Figure 2.2b). I used the natural moth retention pattern to generate expected values for my hand pollinated patterns. I then compared the expected pattern with that observed for both the 1 and ¼ SAL treatments. Both intrinsic patterns derived from hand pollination are significantly different from the extrinsic moth pattern (1 SAL: Contingency $\chi^2=93.951$, df=2, $p<0.001$, ¼ SAL: Contingency $\chi^2=72.828$, df=2, $p<0.001$).

Something about how yucca moths interact with yuccas changes observed intrinsic patterns of fruit set. In the absence of moths, flowers with the lowest ranks contribute most to fruit set, but in the presence of moths the most successful flowers are the middle ranked flowers. However, under both moth and hand pollination, flowers from the final third contribute least to fruit set. By comparing the intrinsic and extrinsic patterns I demonstrated that the interaction with yucca moths changes which flowers become fruit.

The extrinsic moth pollination pattern for *Y. kanabensis* in the present study is most similar to the results reported by Sutherland (1987) or Huth and Pellmyr (1997), where flowers from the middle of the inflorescence or blooming period had the greatest chance of retention. However, the intrinsic pattern resulting from the hand pollination, moth exclusion experiment is most similar to Aker (1982) with more fruit resulting from early, proximal flowers. Two other studies have also considered fruit retention patterns with hand pollinations and moth exclusion (James *et al.* 1994, Huth and Pellmyr 1997) but neither of these studies documented the strong shift in fruit retention that I have described between moth pollinated plants and hand pollinated plants.

The extrinsic pattern of fruit set in *Y. kanbensis* is likely a result of the combination of the intrinsic pattern to mature early flowers and the plants ability to discriminate against flowers that have low levels of pollen and large numbers of ovipositions (Pellmyr and Huth 1994, 1995, Richter and Weis 1995, Addicott unpubl., and see Chapter 3). The shift from the preference for flowers from the first third of the inflorescence (intrinsic) to the middle third of flowers (extrinsic) has several possible explanations relating to moth pollination and oviposition behavior. First, moths may ignore inflorescences early in flowering because small floral displays do not attract them. However, this seems unlikely for these populations because the moth densities and overall visitation rates are very high (55-80% visitation, Addicott 1998). Another, more likely, explanation is that yucca moths may not be equally attracted to and pollinate the first third of flowers because of their behavioral preference for visiting the highest fresh flowers on each inflorescence (Wilson and Addicott 1998). This behaviour would in fact allow moths to preferentially visit wave 1 flowers because the wave 1 flowers always occur higher on the inflorescence than the simultaneous flowering wave 2 flowers. However the preference for yuccas to mature wave 1 flowers is balanced against the intrinsic tendency of *Y. kanabensis* to mature flowers early in the flower opening sequence. Thus fruit at the middle of the inflorescence may reflect that balance between moth behavior to seek the highest flowers and the plants tendency to mature flowers that are pollinated early in the flowering sequence.

2.4 CONCLUSION

The present study did not find any evidence to support the hypothesis that patterns in fruit retention offer *Y. kanabensis* any mechanism to reduce costs of larval feeding and increase plant fitness. Therefore, if cost to the yucca is measured in the number of seeds. eaten, the positions that fruit occupy do not alter this cost and the plant does not regulate its partner through fruit retention patterns. However, the only aspect of the non-random patterns and their potential fitness consequences that were assessed in the present study was vertical position on the inflorescence. After the initial experiments were analyzed it became clear that the differences in flower waves is relevant. Another feature that should be examined is the difference in fitness potential between fruit formed from flowers of different waves.

Table 2.1. Summary of the number of flowers and the number of fruit produced by 30 yucca inflorescences (15 received 1 SAL pollen and 15 received 1/4 SAL pollen). Flowers broken down by wave. Flower wave is determined by the flowering sequence of buds that originate from the same axil position.

Pollen Treatment	**Flower Wave**	**# of Flowers**	**# of Fruit**	**Retention**
1 SAL	1	753	140	0.186
	2	452	32	0.071
	3	49	1	0.020
	subtotal	*1254*	*173*	*Mean=0.138*
1/4 SAL	1	732	124	0.169
	2	478	46	0.096
	3	50	3	0.060
	subtotal	*1260*	*173*	*Mean=0.137*
	Total	*2514*	*346*	*Mean=0.138*

Table 2.2. Logistic regression was used to examine the effect of flower wave on flower fate. Analyses for both 1 and 1/4 SAL pollen treatments are shown. 1 SAL: Overall Model Chi-Square=42.212, df=1, p<0.0001, n=1254; ¼ SAL: Overall Model Chi-Square=16.462, df=1, p<0.001 n=1260.

Term	Coefficient	*df*	Significance
1 SAL Pollen			
Flower Wave	-1.1127	1	p<0.0001
Constant	-0.3608		
¼ SAL Pollen			
Flower Wave	-0.6317	1	p=0.0001
Constant	-0.9626		

Table 2.3. Summary of the proportion of flowers that became fruit from the first, middle and last third of flower ranks. Two different flower ranking methods are included and the results from the ¼ and 1 SAL treatments are listed separately.

	Pollen Treatment	**Rank Group**	**# of Flowers**	**# of Fruit**	**Retention**
Flowers ranked by axil.	1 SAL	1	405	59	0.146
	1 SAL	2	515	90	0.175
	1 SAL	3	334	24	0.071
	1/4 SAL	1	404	69	0.171
	1/4 SAL	2	499	76	0.152
	1/4 SAL	3	357	28	0.078
Flowers ranked by opening date & axil.	1 SAL	1	407	104	0.256
	1 SAL	2	424	47	0.111
	1 SAL	3	422	22	0.052
	1/4 SAL	1	409	93	0.227
	1/4 SAL	2	428	63	0.147
	1/4 SAL	3	424	17	0.040
	Total		*2514*	*346*	*Mean=0.138*

Table 2.4. Logistic regression was used to examine the effect of flower axil position on flower fate. Analyses for both 1 and 1/4 SAL pollen treatments are shown. 1 SAL: Overall Model Chi-Square=9.276, df=1, p<0.0023, n=1254; ¼ SAL: Overall Model Chi-Square=10.668, df=1, p<0.0011 n=1260.

Term	**Coefficient**	***df***	**Significance**
1 SAL Pollen			
Flower Axil Position	-0.0166	1	p<0.0029
Constant	-1.4030		
¼ SAL Pollen			
Flower Wave	-0.0186	1	p=0.0014
Constant	-1.3745		

Table 2.5 Logistic regressions of relative flower rank on flower fate. Relative rank was used to account for differences in the number of axils between plants and incorporated flower opening date, flower axil position and flower wave into the ranking. Analyses for both 1 and 1/4 SAL pollen treatments are shown. 1 SAL: Overall Model Chi-Square=67.959, df=1, p<0.0001, n=1254; ¼ SAL: Overall Model Chi-Square=49.054, df=1, p<0.0001 n=1260.

Term	**Coefficient**	***df***	**Significance**
1 SAL Pollen			
Flower Wave	-2.4920	1	p<0.0001
Constant	-0.7508		
¼ SAL Pollen			
Flower Wave	-2.0776	1	p<0.0001
Constant	-0.9147		

Table 2.6. ANOVA of the seed wet weight, seed dry weight and seed total for fruit from three positions on the yucca inflorescence. Blocking was done by clone.

Source	DF	*F*	Significance	Power
Seed wet weight				
Position	2	1.188	0.353	0.192
Clone	4	14.263	0.001	0.997
Seed dry weight				
Position	2	1.405	0.300	0.220
Clone	4	12.189	0.002	0.992
Seed total				
Position	2	0.827	0.471	0.146
Clone	4	1.843	0.214	0.343

Table 2.7. ANOVA of the average number of seeds consumed per larva from fruit at three different positions on the yucca inflorescence.

Source	**DF**	***F***	**Significance**	**Power**
Position	2	4.053	0.043	0.614
Error	13			

Table 2.8. A summary of reported fruit retention patterns for a number of yucca species and a closely related agave.

Study	Species	Fruit Retention Pattern
Aker, 1982, Aker & Udovic, 1981	*Yucca whipplei*	Lower 1/3 of flowers initiate and result in more fruits than upper 1/3.
Powell & Mackie 1966	*Yucca whipplei*	'...tendency for concentration of capsule development toward the centre of the inflorescence...'
James *et al.* 1994	*Yucca elata*	'Mature fruits were produced at many positions on an infructescence, and were not clumped in any particular region.' Late flowers less likely.
Huth & Pellmyr 1997	*Yucca filamentosa*	'...proportion of flowers retained from first 4 days low,... flowers opening between day 6 and 12 have greatest proportion retained...'
Sutherland 1987	*Agave mckelveyana*	Fruit set is highest in the middle of the flowering stalk.
Present study	*Yucca kanabensis*	Middle ranked flowers most successful with moth pollination but low ranked flowers with hand pollination

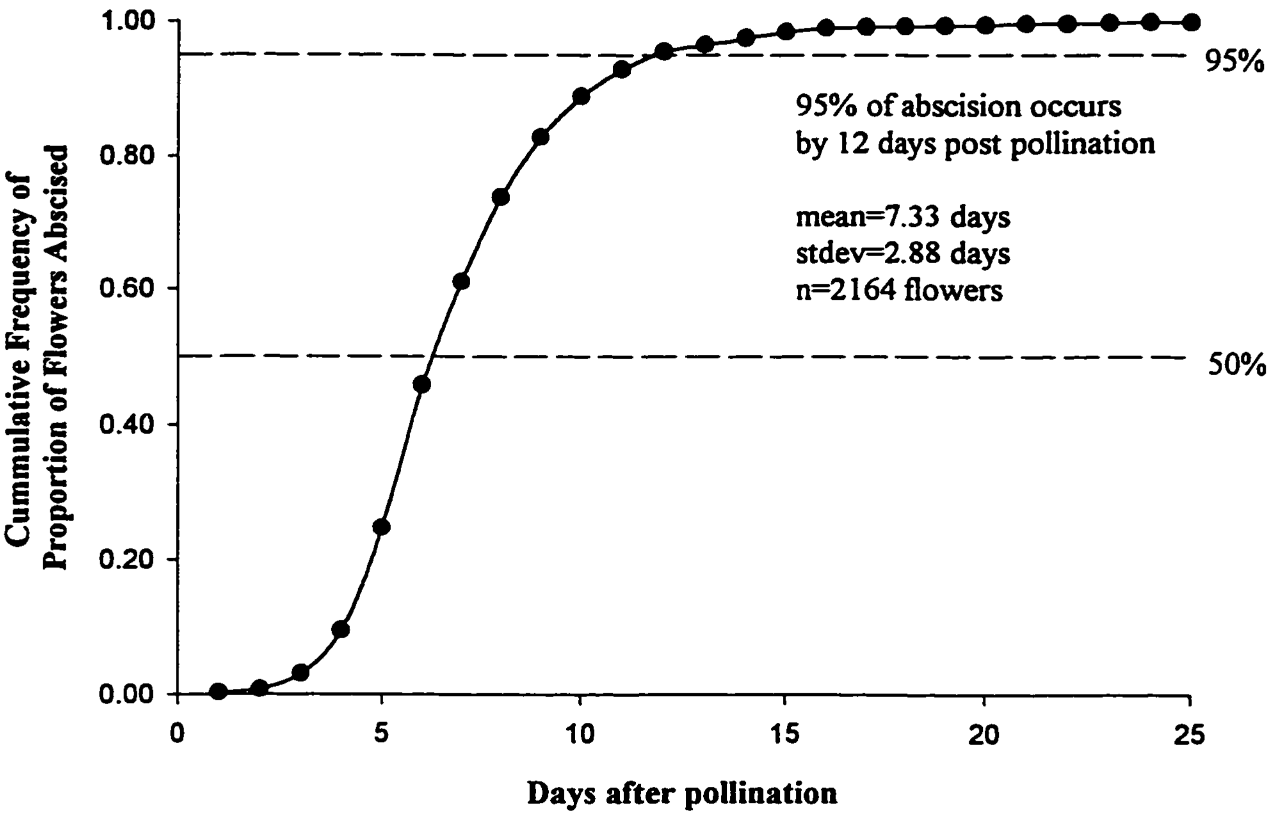


Figure 2.1 The frequency distribution of yucca flower abscision after pollination.

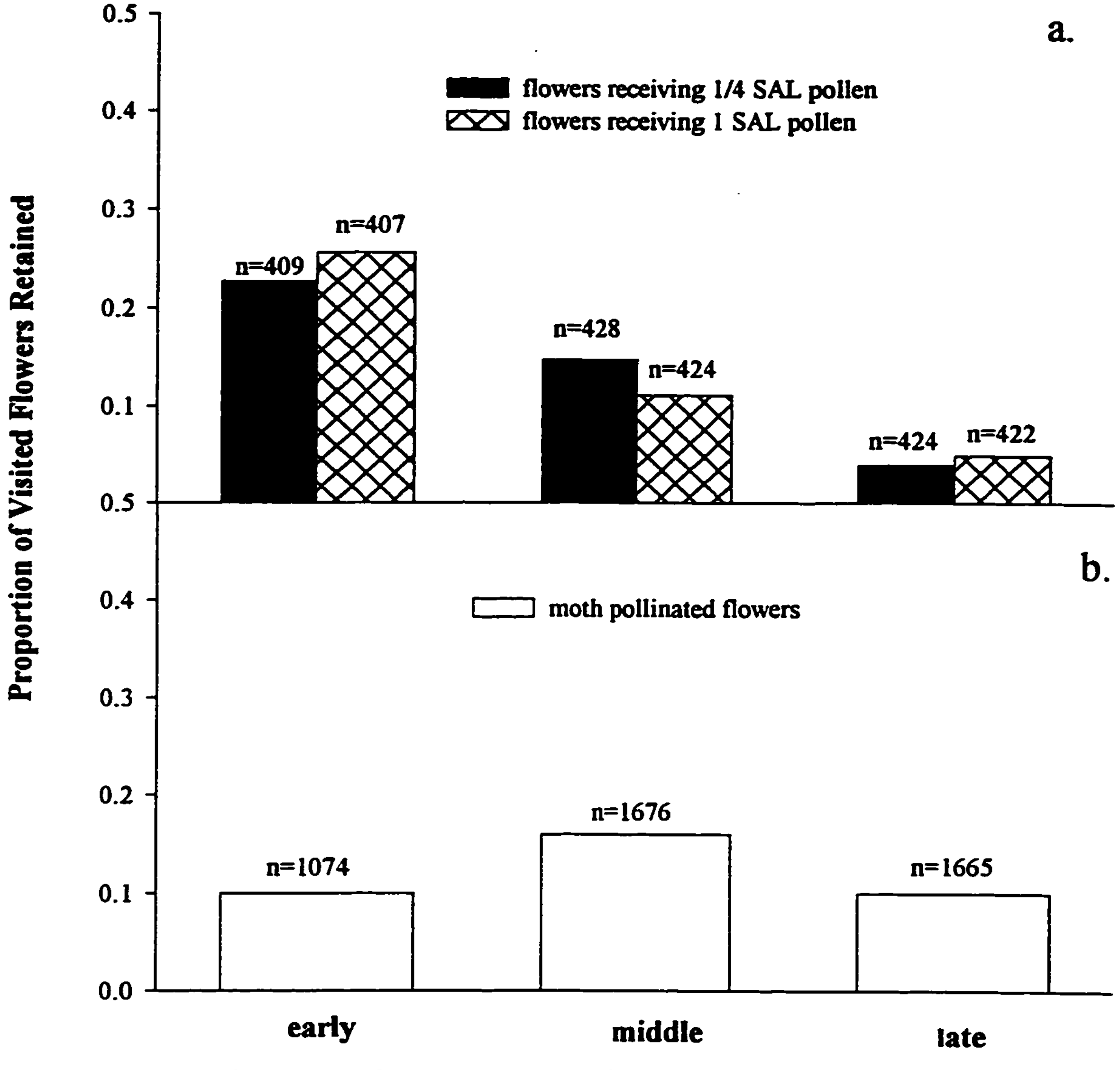


Figure 2.2 Comparison of the proportion of flowers becoming fruit from (a) hand pollinated plants with two different pollen levels and (b) moth pollinated plants. Flowers on each inflorescence were ranked first by opening date and then by axil position.

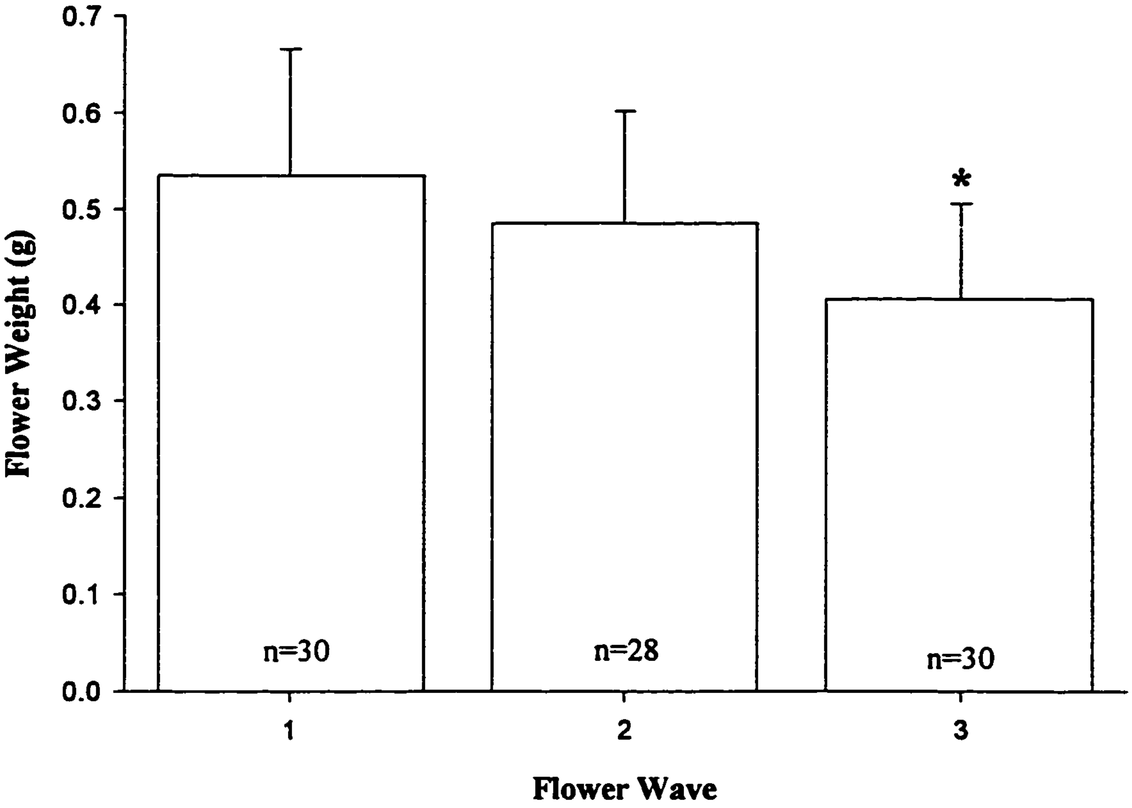


Figure 2.3 Flower dry weights (mean +/- standard deviations) for flowers from the three flowering waves. Asterix indicates which group is significantly different Post-hoc LSD) (ANOVA F= 9.523 df=2 p<0.0001).

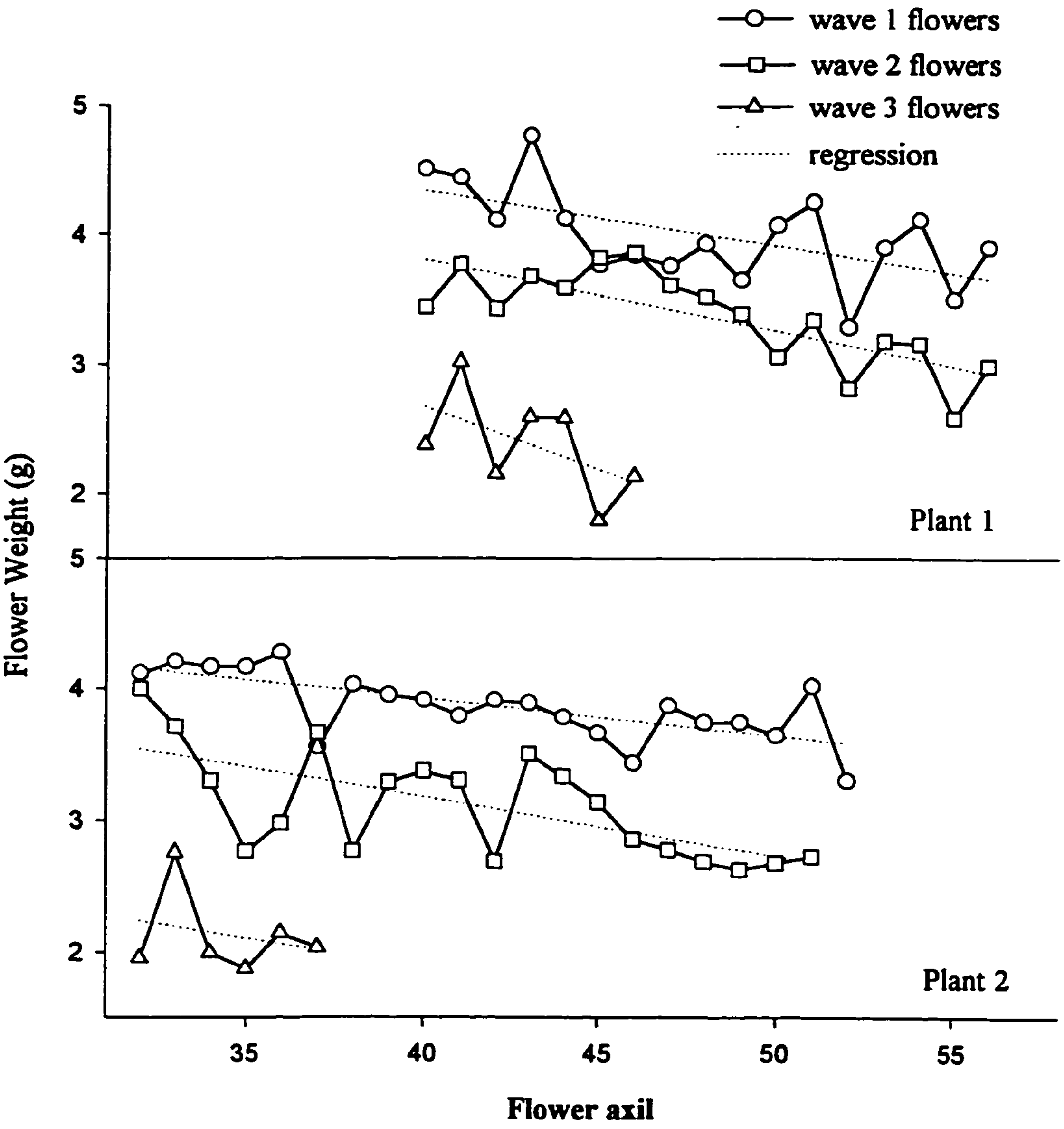


Figure 2.4 Flower weights from wave 1, 2 and 3 flowers collected from consecutive axil positions for two different plants. Plant 1: wave 1 slope=-0.0425, r^2=0.337, df=1, p=0.015; wave 2 slope=-0.0547, r^2=0.59, df=1, p=0.003; wave 3 slope=-0.0975, r^2=0.282, df=1, p=0.368. Plant 2: wave 1 slope=-0.0288, r^2=0.475, df=1, p=0.0005; wave 2 slope=-0.0447, r^2=0.422, df=1, p<0.001; wave 3 slope=-0.0443, r^2=0.067, df=1, p=0.621.

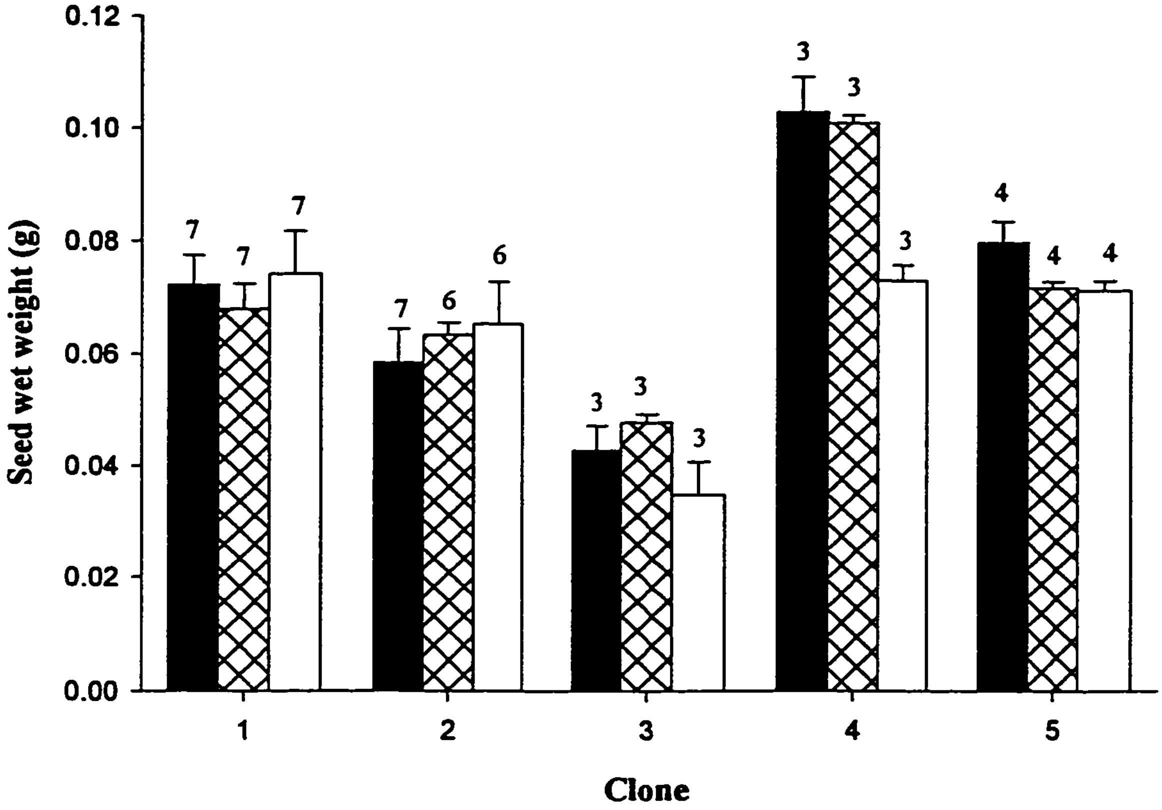


Figure 2.5 Mean wet weight per seed in grams for each position treatment within a clone. Error bars are standard deviations. There are no significant differences between fruit when interclonal variation is accounted for. Numbers above the bars are the number of fruit the mean values were calculated from. Black bars are fruit from the bottom, hatched bars are fruit from the middle, and white bars are fruit from the top.

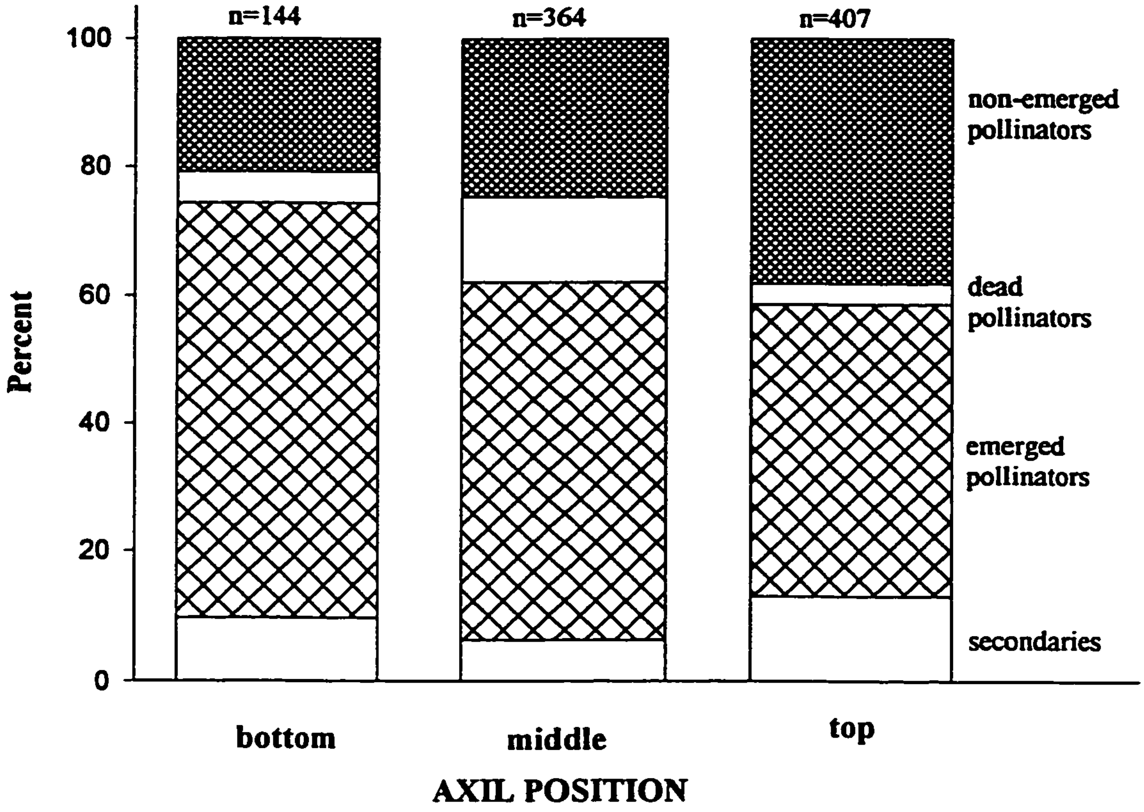


Figure 2.6 Summary of the types and fates of larvae found in yucca fruit obtained from the bottom, middle and top of yucca infloresences.

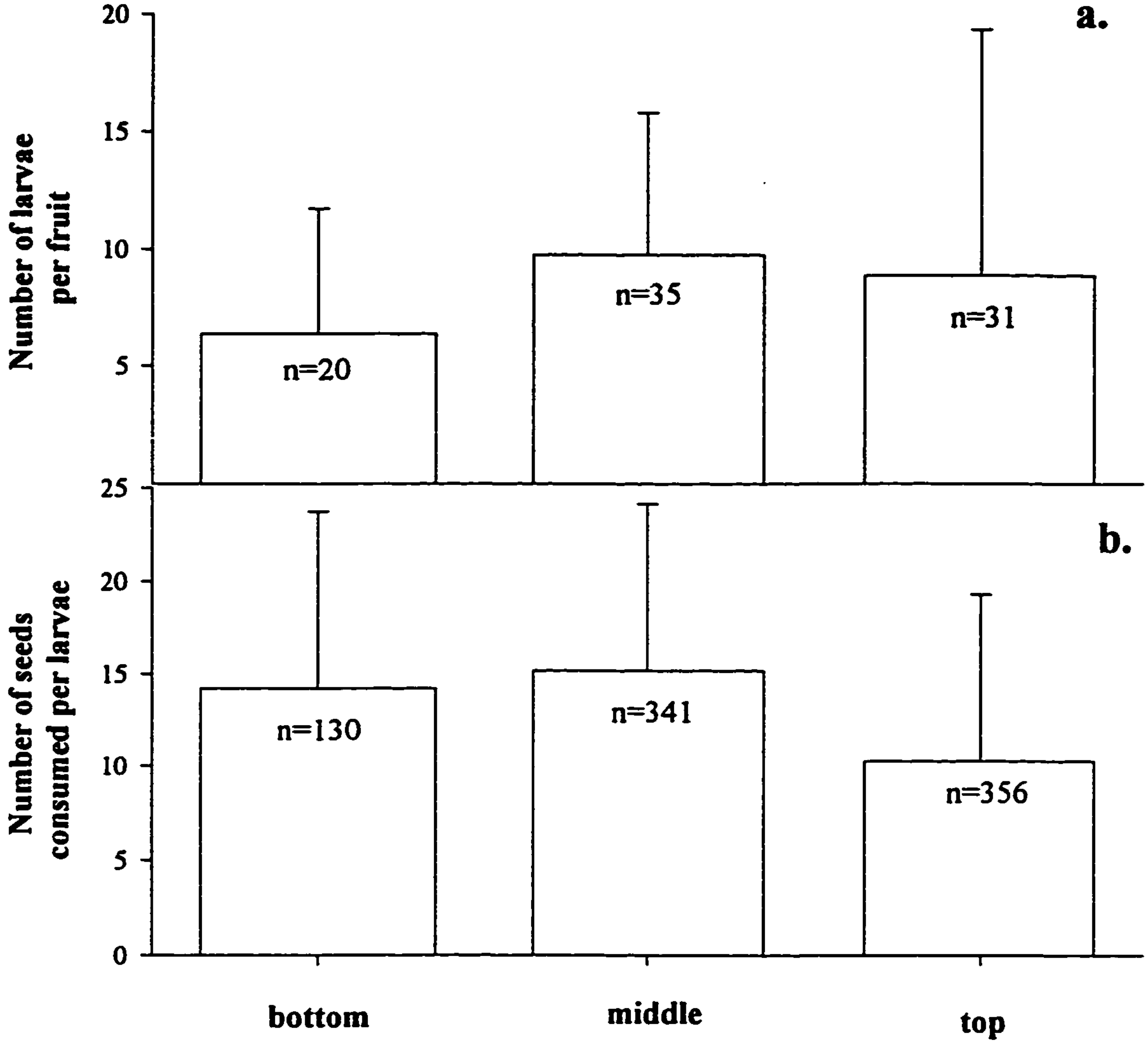


Figure 2.7 (a) Number of pollinator larvae per fruit and (b) number of seeds consumed per larvae (b) from yucca fruit obtained from the bottom, middle and top of yucca inflorescences. Values are means +/- sd.

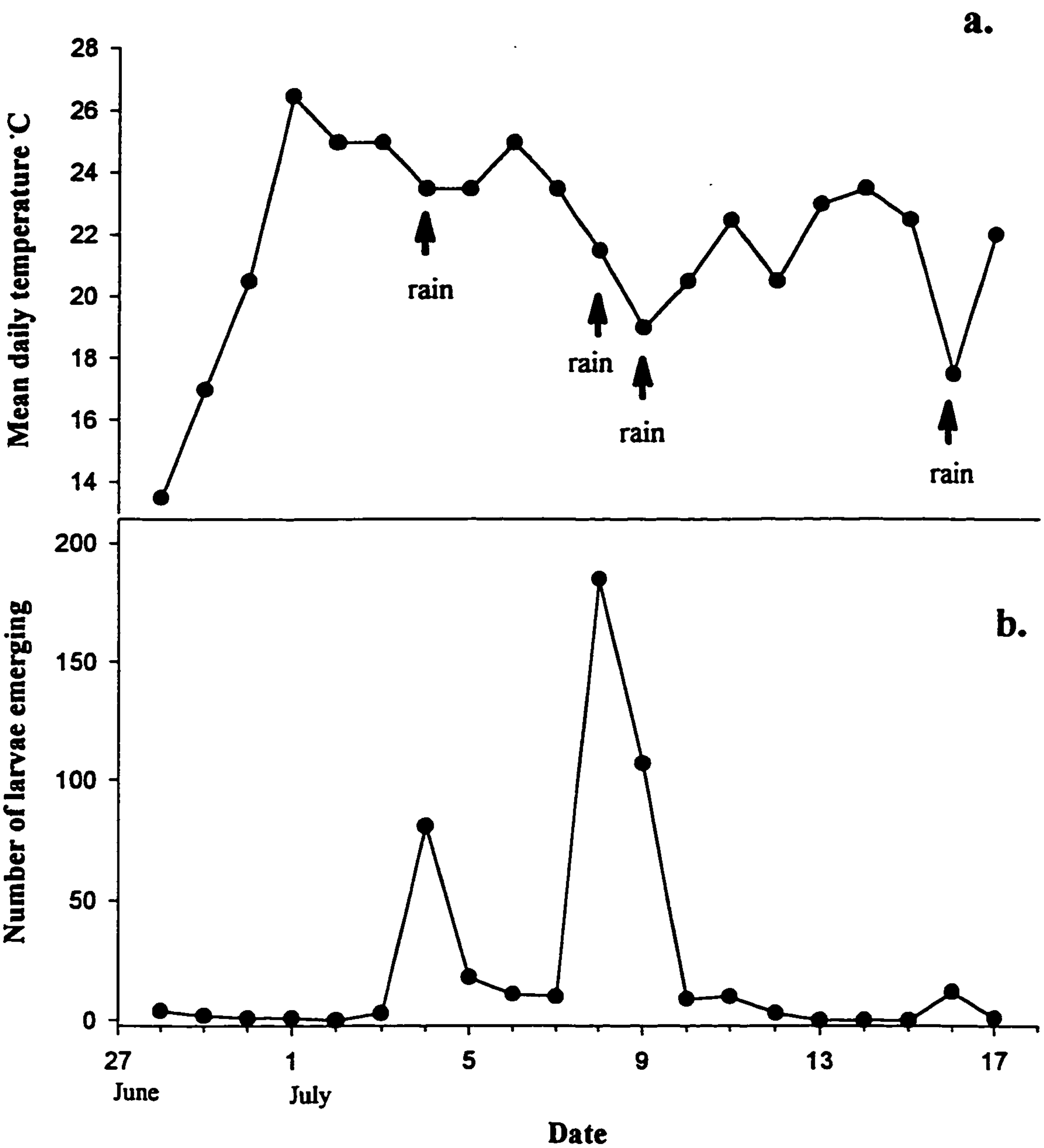


Figure 2.8 (a) Mean daily temperatures and (b) larval emergence between June 28 and July 17, 1996.

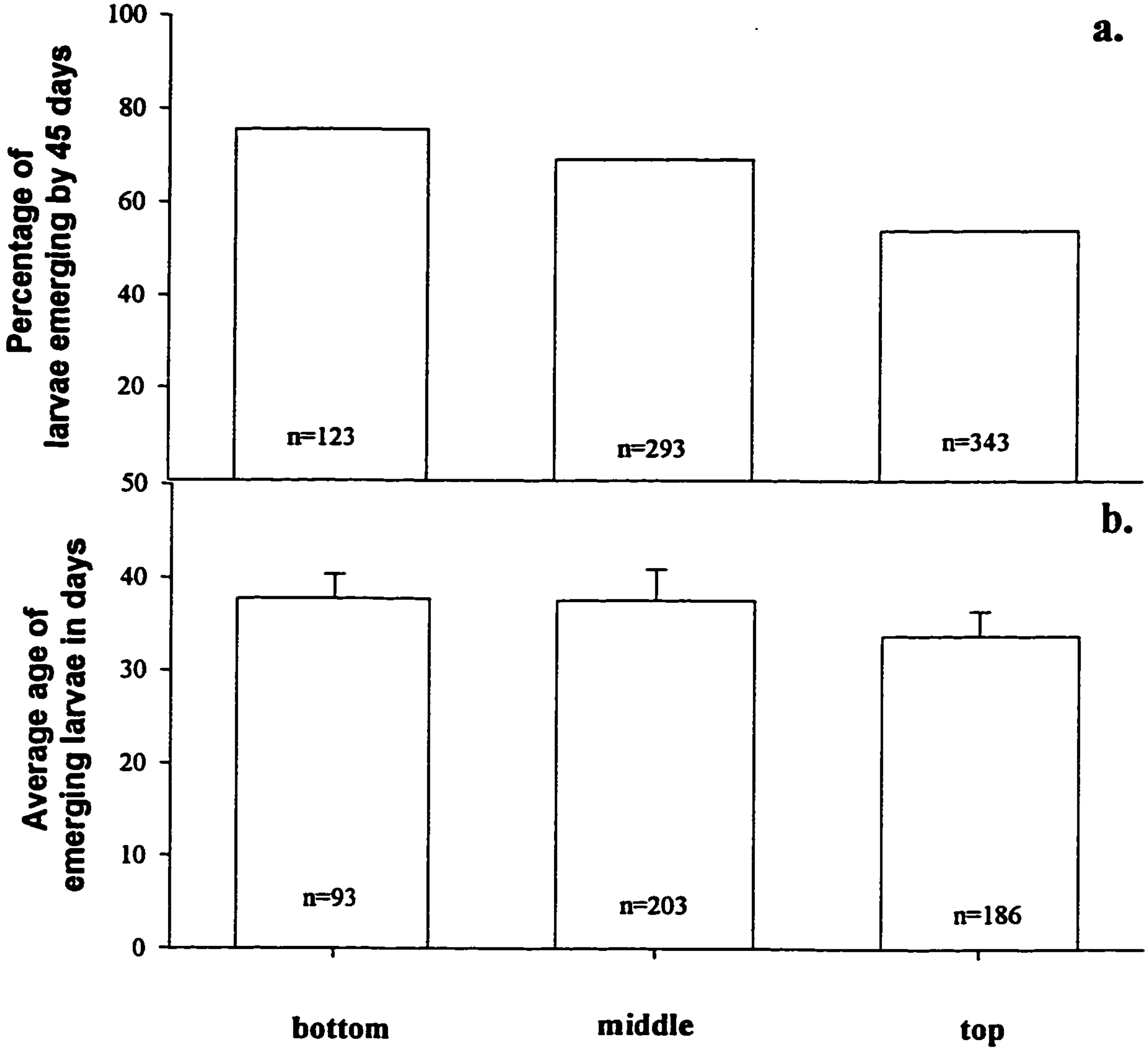


Figure 2.9 (a) The percentage of emerging larvae and (b) the average age of the emerging larvae (b) from yucca fruit obtained from the bottom, middle and top of yucca inflorescences. Error bars are standard deviations.

2.4 LITERATURE CITED

Addicott, J. F. 1986. Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. Oecologia **70**:486-494.

Addicott, J. F. 1996. Cheaters in yucca/moth mutualism. Nature **380**:114-115.

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. Oikos **81**:119-129.

Addicott, J. F., and A. J. Tyre. 1995. Cheating in an obligate mutualism: how often do yucca moths benefit yuccas? Oikos **72**:382-394.

Aker, C. L. 1982. Regulation of flower, fruit, and seed production by a monocarpic perennial, *Yucca whipplei*. Journal of Ecology **70**:357-372

Aker, C. L. and D. Udovic. 1981. Oviposition and pollination behavior of the yucca moth, *Tegeticula maculata* (Lepidoptera:Prodoxidae), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). Oecologia **49**:96-101.

Baker, H. G. 1986. Yuccas and yucca moths - a historical commentary. Annals of the Missouri Botanical Gardens **73**:556-564.

Bawa, K. S. and C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. American Journal of Botany **71**:736-751.

Becerra, J. X. and D. G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormia tenax* (Agaraceae): a second action of self-incompatibility at the whole flower level? Evolution **46**:458-469.

Berry, P. E. and R. N. Calvo. 1991. Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). Plant Systematics and Evolution **174**:93-101.

Cronquist, A., A. H. Holmgren and N. H. Holmgren. 1977. Intermountain flora: vascular plants of the Intermountain West, U.S.A. The monocotyledons, Vol. 6. Columbia University Press. New York.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. Annual Review of Ecology and Systematics **26**:531-552.

Dodd, R. J. and Y. Linhart. 1994. Reproductive consequences of interactions between *Yucca glauca* (Agavaceae) and *Tegeticula yuccasella* (Lepidoptera) in Colorado. American Journal of Botany **81**:815-825.

Goldingay, R. L. and R. J. Whelan. 1993. The influence of pollinators on fruit positioning in the Australian shrub *Telopea speciosissima* (Proteaceae). Oikos **68**:501-509.

Guitian, J. 1994. Selective fruit abortion in *Prunus mahaleb* (Rosaceae). American Journal of Botany **81**:1555-15558.

Harder, L. D., J. D. Thompson, M. B. Cruzan, and R. S. Unnasch. 1985. Sexual reproduction and variation in floral morphology in an ephemeral lily *Erythronium americanum*. Oecologia **67**:286-291.

Herrera, J. 1991. Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Laminaceae). American Journal of Botany **78**:789-794.

Holtsford, T. P. 1985. Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. American Journal of Botany **72**:1687-1694.

Huth, C. J. and O. Pellmyr. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. Oikos **78**:576-584.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1993. Pollination ecology of *Yucca elata*: an experimental study of a mutualistic association. Oecologia **93**:512-517.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1994. Fruit abortion in *Yucca elata* and its implication for the mutualistic association with yucca moths. Oikos **69**:207-216.

James, M. L. 1998. Limits on the exploitation of the yucca-yucca moth mutualism. MSc Thesis, Univeristy of Alberta, Edmonton, Canada.

Lovett Doust, L., J. Lovett Doust, and K. Turi. 1986. Fecundity and size relationships in Jack-in-the-Pulpit, *Arisaema triphyllum* (Araceae). American Journal of Botany **73**:489-494.

McKelvey, S. D. 1947. Yuccas of the Southwestern United States, Part 2. The Arnold Arboretum of Harvard University, Jamaica Plain, MA.

Obeso, J. R. 1993a. Seed mass variation the perennial herb *Asphodelus albus*: sources of variation and position effect. Oecologia **93**:571-575.

Obeso, J. R. 1993b. Selective fruit and seed maturation in *Asphodelus albus* Miller (Liliaceae). Oecologia **93**:564-570.

Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature **372**:257-260.

Pellmyr, O., and C. J. Huth. 1995. Differential abortion in the yucca. Nature **376**:558.

Pellmyr, O., J. N. Thompson, J. M. Brown and R. G. Harrison. 1996. Evolution of pollination and mutualism in the yucca moth lineage. The American Naturalist **148**:827-847.

Powell, J. A. 1984. Biological interrelationships of moths and *Yucca schottii*. University of California Publications in Entomolgy **100**:1-93.

Powell, J. A. and R. A. Mackie. 1966. Biological interrelationships of moths and *Yucca whipplei* (Lepidoptera: Gelechiidae, Blastobasidae, Prodoxidae) University of California Publications in Entomolgy **42**:1-59.

Rau, P. 1945. The yucca plant, *Yucca filamentosa*, and the yucca moth, *Tegeticula (Pronuba) yuccasella* (Riley): an ecologico-behavior study. Annals of the Missouri Botanical Gardens **32**:373-394.

Richter, K. S. 1995. The pollination of *Yucca whipplei* by *Tegeticula maculata*: the benefits and costs of an obligate mutualism. Ph.D. Dissertation. University of California, Irvine. USA.

Richter, K. S. and A. E. Weis. 1995. Differential abortion in the yucca. Nature **376**:557-558.

Riley, C.V. 1872. The fertilization of the yucca plant by *Pronuba yuccasella*. Canadian Entomologist **4**:182.

Riley, C. V. 1892. The yucca moth and yucca pollination. Annual Report of the Missouri Botanical Garden **3**:99-159.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253-279.

Stephenson, A. G., B. Devlin, and J. B. Horton. 1988. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Curcurbita pepo* (Zucchini squash). Annals of Botany **62**:653-661.

Sutherland, S. 1987. Why hermaphroditic plants produce many more flowers than fruits: experimental tests with *Agave mckelveyana*. Evolution **41**:750-759.

Webber, J. M. 1953. Yuccas of the southwest. US Department of Agriculture, Agricultural Monographs **17**:1-93.

Wilson, R. D., and J. F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos **81**:109-118.

Winn A. A. 1991. Proximate and ultimate sources of within-individual variation in seed mass in *Prunella vulgaris* (Lamiaceae). American Journal of Botany **78**:838-844.

Wolfe, L. M. 1992. Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae)?: developmental constraints vs. resource limitation. American Journal of Botany **79**:1286-1290.

Wyatt, R. 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. American Journal of Botany **69**:585-594

Zar, J. H. 1996. Biostatistical analysis 3rd Ed. Prentice Hall, New Jersey.

Ziv, Y. and J. L . Bronstein. 1996. Infertile seeds of *Yucca schottii*: a beneficial role for the plant in the yucca-yucca moth mutualism? Evolutionary Ecology **10**:63-76.

Chapter 3

Regulation of the Mutualism Between Yuccas and Yucca Moths: Intrinsic and Extrinsic Factors Affecting Flower Retention

3.0 INTRODUCTION

Fruit production of many flowering plants is resource limited with plants initiating more flowers than can be supported as fruit. This apparently wasteful but common strategy in fact appears to serve several different and important adaptive functions. For example, surplus flowers are important for creating large displays for attracting pollinators, allowing plants to take advantage of unexpected resources in good years, increasing male function through pollen donation, and providing insurance against unexpected catastrophes (reviewed by Stephenson 1981). Finally, surplus flowers may allow plants to maximize fitness by selectively maturing flowers of the highest quality (Stephenson 1981). Some flower features known to affect flower (and subsequent fruit) quality and hence whether a flower is matured or abscised include: flower position on the inflorescence (Wyatt 1982, Sutherland 1987, Herrera 1991, Guitian 1994, Brunet 1996), flower timing and flowering history (Holtsford 1985, Stephenson *et al.* 1988, Ehrlen 1993, Guitian *et al.* 1996), the amount of pollen (Winsor *et al.* 1987, Stephenson *et al.* 1988, Schlichting *et al.* 1990, Young and Young 1992) and the source of pollen received (Cruzan 1990, Becerra and Lloyd 1992).

Yucca (*Yucca* spp., Agavacea) pollination is performed by one or more members of an unresolved complex (Addicott and Tyre 1995, Pellmyr *et al.* 1997) of closely related moths in the genus *Tegeticula* (Incurvariidae). The obligate mutualism was first described in detail by Riley (1872) and has been the subject of many recent studies (Aker and Udovic 1981; Powell 1992; James *et al.* 1993; Addicott and Tyre 1995; Pellmyr *et al.* 1996; Ziv and Bronstein 1996). Female yucca moths have a number of behavioral and physical adaptations that allow them to actively pollinate yuccas and they also lay their eggs inside of the flowers where the developing moth larvae become seed predators. Neither species can reproduce sexually in the absence of the other partner (but see Dodd and Linhart 1994).

However, the apparent co-operation between yuccas and yucca moths is a balanced conflict (Fuller 1990) between plants' needs to produce seeds and moths' needs to produce larvae. This mutualism is of continued interest because it is one of the best vehicles for the study of the fitness conflict between the partners and its subsequent resolution through partner regulation (Addicott and Bao in review). One element that is important to the regulation of the conflict is the flowering/fruiting strategy of the yucca. Although the surplus flowering and limited fruit production of yucca is not a particularly unique or unusual feature, it likely played a role in the initial evolution (Pellmyr *et al.* 1996) and continued existence of this insect plant relationship.

I studied the surplus flowering strategy of one species of yucca that typically matures around 10% of initiated flowers (Addicott and Tyre 1995), abscising the rest. Some sources of variation in flower quality are the same for *Yucca kanabensis* McKelvey as they would be for other flowering plants. These include differential flower quality based on variation in flower position on the inflorescence and in the flowering sequence (see Chapter 2), as well as differences in the amount and sources of pollen that flowers receive. In addition, due to the unique nature of yucca pollination, the density of eggs and larval feeding of yucca moths also influence flower quality.

I assumed that the function of surplus flowers in *Y. kanabensis* was to allow the plant to mature those flowers of the highest quality. Given the unique pollination/seed predation relationship, flower quality in *Y. kanabensis* has two components. The first is extrinsic to the plant but controlled by the moth, and includes the number of eggs that moths have laid in a flower and the quality of the pollination service performed (i.e. pollen quantity, freshness and genetic compatibility). Many of the studies on yucca flower retention have focused on these extrinsic moth mediated effects (Tyre and Addicott 1993, Pellmyr and Huth 1994, Pellmyr *et al.* 1997, Wilson and Addicott 1998). However, extrinsic moth mediated effects do not completely explain flower retention, indicating that a second, intrinsic component to flower quality may exist. Further, field observations of non-random fruit retention in *Y. kanabensis* (e.g. Addicott 1998) and *Y. elata* (e.g. James *et al.* 1994) that are unexplained by moth visitation indicates the possibility of other factors.

This second component of flower quality is intrinsic to plants, relating to inflorescence architecture and phenology.

Yucca kanabensis has a large, unbranched inflorescence that flowers acropetally (from bottom to top) in a series of two or three waves. The relatively uncomplicated structure and phenology of this species makes it ideal for studying flowering and fruiting patterns and how these patterns are affected by ovipositions, pollination, and intrinsic flower position and timing effects. The present study attempts to tease apart the intrinsic and extrinsic factors affecting flower success and then recombine these factors in a controlled way to understand how the factors interact with each other.

I address five specific questions. First, does *Y. kanabensis* selectively abscise surplus flowers based solely on the amount of pollen received? Second, does the order of presentation of different amounts of pollen affect which flowers become fruit? Third, do flowers from different flowering waves interact in the same way with pollen levels? Fourth, is there an interaction between flowers from different waves with different pollen loads? Finally, what is the combined contribution of pollen, flower position, wave effects and known oviposition effects to flower success?

I will show that flower retention is influenced by an interplay between intrinsic and extrinsic factors and how this has consequences for both plant and moth fitness and moth behavior. It is ultimately these interactions which control costs and benefits that both the plant and the pollinator receive and will further out understanding of the conditionality and regulation of this unique mutualism.

3.1 METHODS

3.1.1 Study Site

I performed a series of experiments during the 1996 and 1997 flowering season at two study sites near Kanab, Kane County, Utah, USA. One site was located on the road to Coral Pink Sand Dunes State Park (112° 40' 45" W, 37° 7' 30" N, 1800 m elevation) near

Yellowjacket Ranch and the other on the north side of US Highway 89 on the sandy ridge between Kanab and Mt. Carmel Junction (112° 36' 30" W, 37° 10' 15" N 1838 m elevation). *Yucca kanabensis* McKelvey is commonly scattered amongst the sage (*Artemesia tridentata* Nutt.), juniper (*Juniperus monosperma* Engelm.), and Gambel oak (*Quercus gambellii* Nutt.) at these sites (see Addicott and Tyre 1995).

3.1.2 Study Organisms

Yucca kanabensis McKelvey has narrow pointed leaves and forms discrete basal rosettes. It forms large clonal clumps through vegetative growth, but establishment from seed is also common. Individual rosettes are long lived and do not flower every year. Flowering at these sites in southern Utah generally occurs between late May and early July. The inflorescence is an unbranched raceme composed of 30-70 flower bearing nodes or axils. Each axil position typically contains 1-3 flower buds that belong to different waves of flowering. At each axil the buds from different waves are at different stages of maturity and do not open together but are staggered in development by an average of 3.8 days (sd=1.28 n=899 paired flowers). When flowering commences it is acropetalous (beginning at the bottom and moving upwards), beginning with the first wave of flowers at each axil position. Approximately 4 days after wave 1 flowering begins, but well before the entire first wave of flowers open, the second wave of flowering begins. Thus, near the midpoint of flowering an individual plant may have fresh wave 1 flowers near the top, wave 2 flowers near the middle and wave 3 flowers (if they occur) near the bottom. Each flower is perfect and is both attractive to moths and receptive to pollen for two nights. The plant is resource limited, maturing ~10% of flowers into fruit and abscising the rest (Addicott and Tyre 1995). Seeds are mature in ~60 days and are then gradually wind/gravity dispersed.

Two different pollinating members of the *Tegeticula yuccasella* Riley species complex are found at our sites. The experiment described here used only the female moths designated as deeps (Addicott and Tyre 1995). These moths have a longer ovipositor and lay their eggs deeply within the flower pistil tissue along the ovules. The ovipositions of deep moths are known to trigger selective flower abscission at these study sites (Wilson and Addicott 1998). Moths are active at night but rest within flowers during the day where they can be easily found and captured for manipulation or enumeration.

3.1.3 Pollination Protocol

All experiments employed hand pollination of flowers using the following methods: I chose a series of pollen donor plants located more than 500 m from any recipient plants. Initially I removed any open flowers and then enclosed each plant in a long, tubular, zippered bag made of flexible fiberglass window screening and fine netting to exclude moths. On each subsequent morning I removed all fresh flowers and placed them in a cooler until required for pollen transfers. I randomly chose flowers from the cooler, removed the petals, and carefully detached the crude pollinia from each of the anthers with a yucca leaf blade, transferring the mass of pollen to one of the removed petals. Pollen loads were then carefully transferred to the recipient flower and gently placed down the style. No effort was made to ensure that each flower on a recipient plant received pollen from the same or different donors. Results from extensive hand pollination experiments (see Chapter 2) indicated that the numbers of fruit setting were similar to the range seen under moth pollination.

I chose to investigate the effects of two different pollen levels: a low level, equivalent to a quarter of the amount of pollen contained on one anther and a high level, equivalent to the entire amount of pollen contained on one anther. Hence, pollinia were either left intact for the 1 standard anther load (SAL) treatments or divided into roughly equal quarters for the ¼ SAL treatments. The low pollen level is similar to the average amount of pollen flowers receive from moth pollination and the second high level is similar to the heaviest pollen levels naturally pollinated flowers receive (JFA unpublished data). Both pollen levels have enough grains to fertilize all of the ovules in a *Y. kanabensis* flower as each anther has an average of ~4000 pollen grains and each flower has an average of 318 ovules (see Chapter 2).

3.1.4 Experimental Overview

I performed five separate experiments (Figure 3.1). The first three focused on variation in pollen, which is one aspect of extrinsic flower variation that affects flower success. The fourth and fifth experiments then combined both aspects of extrinsic variation, pollen and ovipositions, and investigated how these factors interact with intrinsic flower features to influence flower success.

All experiments utilized only a small subset (~20%) of flowers available on each plant. Similar to Wilson and Addicott (1998) I chose to work with this number of flowers as only ~10-13% of flowers per plant will mature to fruit (Addicott 1998, see Chapter 2). By using only ~ 20 % of flowers, dividing them into two treatments and expecting only about 10 % to eventually be retained provides a more sensitive and efficient protocol to detect differences between treatments than using 100% of flowers and expecting 90% of flowers to be abscised. Preliminary experiments indicated that removal of the excess buds and flowers does not change the total number of flowers that mature as fruit providing the plant is left with at least the 10% that would have originally be retained. In addition, my removals and the subsequent effect on the plant's ability to set fruit are similar to natural removals caused by deer and cattle grazing.

3.1.4.1 Experiments 1 & 2 - Pollen Quantity (Wave 1) & (Wave2)

The pollen quantity experiments were intended to determine if yuccas selectively retain flowers with higher pollen loads, implying that higher pollen loads lead to fruit of higher quality through pollen competition. Within each experiment there were also two pollen sequence treatments. This was done to determine if pollen selectivity for higher pollen loads is influenced by whether higher loads come first or last in the pollination sequence. A separate experiment was done for wave 1 and wave 2 flowers to see if pollen selectivity occurred in both groups of flowers.

Plants for these two experiments were interspersed along the Highway 89 site. For each experiment I chose a non-random sample of yucca clones, including only those clones with more than one flowering stalk and clones with inflorescences that had more than 40 axils. Two stalks were required so that I could repeat the two sequence treatments within the same clone. I bagged each inflorescence prior to the initiation of flowering to exclude deer and pollinators. For both experiments I used only a subset of 20 flowers from the middle third of the flowering stalk. I removed all other flowers prior to the start of flowering. For Experiment 1 I used only wave 1 flowers and for Experiment 2 only wave 2 flowers (Figure 3.1). I randomly assigned inflorescences within experiments to one of two treatments. For treatment 1 plants the first 10 flowers received high pollen and the next 10 flowers received low pollen. For treatment 2 plants the order of high and low pollinations was reversed.

Essentially, flowers were competing against each other based on pollen quantity and order of presentation to see which flowers would be retained. After completing the hand pollination I left the flowers undisturbed until they either abscised or became fruit. I monitored plants daily for 30 days and removed the inflorescence cages a week after pollinating the last flower.

For Experiment 1, I used 8 clones with 2 inflorescences each for a total of 16 inflorescences. However, deer ate all of the immature fruit from three inflorescences. For Experiment 2 deer also ate a number of 1996 replicates so the analysis is based on 4 clones (8 inflorescences) from 1996 and 8 clones (16 inflorescences) from 1997, leaving 12 inflorescences per pollen sequence treatment. I counted and compared the number of fruit on each inflorescence resulting from the two different pollen levels to see if there was a pollen level preference. I analyzed this in two ways, first treating the inflorescence as the unit of investigation and comparing the number of fruit resulting from each pollen level using Wilcoxon-Signed Ranks Paired Samples. I did a separate analysis of the same data sets using logistic regression, which treats each flower as the unit of investigation instead of each inflorescence. Flower fate (abscised/retained) as a function of pollen quantity was analyzed separately for both treatments within both experiments.

3.1.4.2 Experiment 3 - Pollen Quantity/Waves Combined

This experiment was similar to the first two in that it tested whether yuccas selectively abscise flowers on the basis of pollen quantity. However unlike the previous two experiments I used flowers from both waves simultaneously to see the interplay between pollen and flower waves. There is strong intrinsic selection for wave 1 flowers when multiple flower waves are present (see Chapter 2) and I was interested in determining if pollen quantity and order of presentation modify this inherent flower wave preference. I used the same location and essentially the same techniques for Experiment 3 as described above. In the first treatment the first 5 axils of paired wave 1 and 2 flowers were given high pollen and the last 5 axils were given low pollen. For the second treatment this was reversed with the low pollen flowers coming first (Figure 3.1). Again flowers were followed until they became fruit or abscised.

The design of the experiment included eight clones each with two inflorescences for a total of 16 flowering stalks. Eight inflorescences received high pollen first (treatment 1) and eight inflorescences received high pollen last (treatment 2). Logistic regression, with flower fate (abscised or retained) as a function of pollen level and flower wave was used for the analysis. I used backward conditional model fitting with all factors and interactions entered into the initial model.

3.1.4.3 Experiment 4 - Pollen Load Vs Ovipositions

The focus of Experiment 4 was to investigate how the combination of pollen quantity and number of ovipositions influence flower retention. Under natural situations both pollen quantity and number of ovipositions are interrelated but vary considerably. I experimentally decoupled the extrinsic moth mediated effects by administering controlled pollen loads and then allowing normal moth ovipositions. In addition I used the same pollen loads as in the pollen quantity experiments to allow comparisons between the two experiments with and without ovipositions.

This experiment was performed at the Yellowjacket site where the deep moths are the only pollinator present. Fourteen flowering plants were randomly chosen from the population. All wave 2 and 3 buds were removed from these plants and only wave 1 buds were used (Figure 3.1). This was done to simplify the experiment in two ways. First it is known that if there are flowers from all three waves, wave 1 flowers are preferentially retained and contribute most to fruit set (see Chapter 2). Second, variation due to the overlap in flowering sequence between waves could be accounted for by removing all other flowering waves but wave 1. At approximately 7:00 P.M. each night plants were visited and if four flowers were going to open they were randomly assigned both a pollen treatment and an oviposition level treatment, resulting in one flower for each of the four possible treatments: low pollen/low oviposition, low pollen/high oviposition, high pollen/low oviposition and high pollen/high oviposition. If less that 4 flowers were to open they were removed and if more that four flowers were to open they were also removed, unless there were 8 in which case two replicates were done.

To ensure that moths did not pollinate flowers before I did, I started my pollinations in the early evening before the flowers naturally opened. Petals of closed flowers were gently forced apart and flowers were pollinated with their assigned pollen level. After pollination, a small ball of Crayola ® non-toxic non-hardening modeling clay was gently inserted into the style above the pollen to prevent the moths from adding more pollen to the flower. Preliminary attempts with this method showed that the clay neither affects pollen tube growth or fertilization, nor moth oviposition and pollination behavior. In fact, moth pollination behavior appeared unaffected, as indicated by moth deposition of pollen on top of the clay plug.

Female moths that had been collected that morning were then placed in the bag at 2 moths per open flower. The following morning plants were visited and half of the flowers (those assigned to the low oviposition treatment) were covered with small net flower bags. In this way half of the flowers could only be visited on their first night and the other half could be visited on their first and second nights. Flowers from these yucca populations are known to be attractive and receptive on two nights. This procedure was repeated nightly until the plant no longer had enough buds left to open four flowers in one night. Moth levels were also adjusted nightly to account for mortality and escapes while pollinations were being done.

Flowers were then followed and harvested either at 15 days or earlier if flowers naturally abscised. Abscised flowers were collected and dissected to count the number of ovipositions. Harvested 15-day-old fruit were also dissected to determine the number of ovipositions received. Since most natural abscission occurs before 15 days (see Chapter 2 Fig. 2.1) these harvested fruit were considered to be the ones that would have been retained by the plant and fully matured.

To dissect for ovipositions, thin sections of the carpel wall were shaved away with a scalpel to reveal the ovipositor tracks. Tracks were then followed down through thin sections to the ovules. However, no attempt was made to find actual eggs or developing larvae because there is a 1:1 relationship between ovipositions and eggs for this pollinator (Addicott unpublished).

A total of 328 flowers were pollinated but 4 flowers were lost and so all analysis is done on the 324 flowers with complete data. Approximately 20 flowers were used per plant with 14 plant replicates. Of the 324 flowers used in the analysis 42 resulted in fruit and the remainder abscised. Originally it was intended to have two levels (high and low) of two factors (pollen and oviposition), however initial analysis showed that there were no differences in the number of ovipositions between the 'high' and 'low' oviposition treatments (mean=15.45 sd=8.89 and mean=14.64 sd=9.04 respectively) so this variable was treated as continuous. Flower fate as a function of number of ovipositions, flower position by axil and pollen level was then analyzed using logistic regression procedures. Backward conditional model fitting with all factors and interactions entered into the original model was used.

3.1.4.4 Experiment 5 - Wave Vs Ovipositions

The final experiment determined how flower position on the inflorescence and flower wave interacted with ovipositions to determine flower retention. Twelve randomly selected plants at the Yellowjacket site were used for the final experiment. Each plant was stripped of all of its flowers except for the paired wave 1 and wave 2 flowers at 10-14 axil positions near the middle of the inflorescence (Figure 3.1). Each plant was then put in a cage and female moths were added at a rate of 1 moth per fresh flower. Each evening plants were visited and the flowers that were opening were given 1 SAL of pollen. No effort was made to stop the moths from adding additional pollen because there is no selectivity for pollen above 1 SAL (Humphries unpublished data). Flowers were then followed until they abscised or until the last flower to open was 10 days old. When the last flower on each plant was 10 days old all remaining immature fruit were harvested. All abscised flowers and immature fruit were dissected and the number of ovipositions were counted.

A total of 302 flowers distributed among 12 plants were used in this experiment, 151 were wave 1 flowers and 151 were wave 2 flowers. Flower fate (retained/abscised) as a function of ovipositions, flower position and flower wave was then analyzed using logistic regression procedures (backward conditional model fitting).

3.1.5 Statistical Analysis

Data analysis was performed using SPSS for Windows, version 7.5, using the logistic regression test procedures. Non-parametric tests were also done as in Zar 1996.

3.2 RESULTS

3.2.1 Experiment 1 - Pollen Quantity - Wave 1

Pollen quantity experiments were designed to determine if yuccas selectively retained flowers with higher pollen loads. Experiment 1 used only wave 1 flowers and had two pollen presentation treatments (high pollen first and high pollen last). A greater proportion of high pollen flowers were retained as fruit than low pollen flowers for both sequences of pollen delivery (Figure 3.2). High pollen flowers were approximately 40-50% more likely to be retained than low pollen for both pollen presentation treatments. These results were significant when analyzed at the inflorescence level (Wilcoxon Signed Ranks Paired Samples, (T+=19.5, T-=0, p=0.05 n= 6 pairs) and at the flower level where pollen load was a significant predictor of flower success (Logistic Regression-Treatment 1 pollen load B=0.8421, Wald 5.156, df=1, p=0.0232; Treatment 2 pollen load B=0.1078, Wald 8.7065, df=1, p=0.0032). *Y. kanabensis* preferentially retained wave 1 flowers with higher pollen loads over wave 1 flowers with low pollen loads whether the high pollen loads occur first or last in the sequence.

3.2.2 Experiment 2 - Pollen Quantity - Wave2

The second pollen quantity experiment focused on pollen selectivity among wave 2 flowers. For plants where high pollen came before low pollen the results were similar to the pollen selectivity results of the wave 1 flowers, with retention of high pollen flowers being 40% greater than low pollen flowers (Figure 3.2). This indicates a significant selection for high pollen flowers when they occur first whether the data is analyzed at the level of the plant (Wilcoxon-Signed Ranks Paired Samples, T+=77, T-=9.5, p=0.05, n=12 pairs) or the flower (Logistic Regression-Treatment 1 pollen load B=0.8706, Wald 12.895, df=1, p=0.0003). However where high pollen flowers were preceded by low pollen flowers, there was no significant difference in the proportion of fruit setting from either high or low pollen (Figure 3.2). This was analyzed at the level of the plant (Wilcoxon-Signed Ranks Paired Samples, T+=32.5 T-=23, p=0.05 n=12 pairs) and at the level of the flower (Logistic Regression-Treatment 2 pollen load B=0.069, Wald 0.0816, df=1, p=0.7751).

For wave 1 flowers, high pollen levels significantly influenced flower retention whether the high pollen flowers occurred before or after low pollen flowers. This contrasts with the results for wave 2 flowers, where there is only significant selection for high pollen flowers when they coincide with those flowers occurring first in the flowering sequence.

3.2.3 Experiment 3 - Pollen Quantity - Waves Combined

The purpose of Experiment 3 was to determine how pollen quantity interacts with flower wave when both waves are present. In particular, I was interested in determining if wave 2 flowers which normally have lower retention than wave 1 flowers might be retained more often if they have higher pollen loads than wave 1 flowers. Again I was also interested whether the order of pollen presentation might have an effect and hence tested high pollen on the wave 1 flowers followed by low pollen on wave 2 flowers and visa versa. To assist in the interpretation of the results I numbered the bars in the figures (Figure 3.3 a & b) to show the sequence of flower opening (1, 2, 3, 4). The early wave 1 flowers open 1st, the late wave 1 flowers open 2nd, the early wave 2 flowers open 3rd and the late wave 2 flowers open 4th. However, since flower opening in each wave is staggered by ~4 days the last wave 1 flowers and the first wave 2 flowers open at approximately the same time.

A comparison was made of the flowers that resulted in fruit and those that were abscised for each of the two pollen presentation treatments. Overall, wave 1 flowers had higher retention than wave 2 flowers for both high pollen first and last (Figure 3.3a & b) but there were differences in the contribution of the waves in response to the order of presentation of pollen treatments so they will be considered separately below.

3.2.3.1 High Pollen to Lower Flowers

Where flowers on the first 5 axils (paired wave 1 and 2 flowers) received high pollen and flowers on the last 5 axils (paired wave 1 and 2 flowers) received low pollen, more fruit were set from the wave 1 flowers than the wave 2 flowers and within each flower wave the higher pollen load flowers were preferentially retained (Figure 3.3a). Logistic regression indicated that both flower wave and pollen level were significant predictors of flower fate (Table 3.1).

For this treatment, the most interesting comparison is between the late opening wave 1 flowers (opening 2nd in the sequence) and the early opening wave 2 flowers (opening 3rd in the sequence) (Figure 3.3a). Late wave 1 flowers have the advantage of belonging to the 1st wave but have lower pollen levels, while early wave 2 flowers have the disadvantage of 2nd wave but the advantage of higher pollen. There is little difference in success of the two groups indicating an important interplay between the wave, pollen and timing.

3.2.3.2 Low Pollen to Lower Flowers

On the plants where low pollen preceded high pollen flowers, a different retention pattern occurred (Figure 3.3b). The interaction between flower wave and pollen was the only significant predictor variable of flower retention (Table 3.2). Under this regime, wave 1 flowers still have a greater contribution to fruit set and within the wave 1 flowers, pollen load is still an important factor with more high pollen flowers becoming fruit. However unlike the previous pollen presentation treatment (high pollen first) there is no pollen selectivity between the wave 2 flowers with about the same proportion of high and low pollen flowers becoming fruit. Thus using logistic regression, the flower wave by pollen interaction is the strongest predictor of flower success (Table 3.2).

Again the comparison between the late wave 1 flowers (opening 2nd in the sequence) and the early wave 2 flowers (opening 3rd in the sequence) which overlap most closely in flowering time is critical. This time, late wave 1 flowers have the compounded advantage of being from the 1st flowering wave and having the high pollen load while the late wave 2 flowers are doubly disadvantaged by belonging to the less successful 2nd wave and having the low pollen level. Therefore it is not surprising that 87% of the section of flowers opening 2nd become fruit while only 34% of the flowers opening 3rd become fruit (Figure 3b).

Therefore, in the absence of yucca moth ovipositions; flower wave, pollen level and timing interact to influence flower success. First, early flowers tend to do better overall than later flowers. Second, for flowers that open at roughly the same time (late wave 1 and early wave 2) the positive effects of first flowering wave and high pollen can compound each other (as in treatment 2), or the positive effect of flowering wave can be cancelled out by low pollen and vice versa (as in treatment 1).

3.2.4 Experiment 4 - Pollen vs. Ovipositions

Experiment 4 was designed to examine the effect of the amount of pollen and the number of ovipositions a flower received on flower retention. Approximately 20 wave 1 flowers per plant were given two controlled levels of pollen and then moths were permitted to oviposit naturally into flowers. Flower success was analyzed using backward elimination logistic regression test procedures. Pollen treatment, axil position, total ovipositions and all interactions were entered into the first step of the model and the best fitting result indicates that the total numbers of ovipositions is the only significant predictor of flower success (Table 3.3). The more ovipositions a flower receives the less likely it will be retained. The effect of ovipositions outweighs the effect of pollen level. Figure 3.4 shows flower retention based on the number of ovipositions that a flower receives.

3.2.5 Experiment 5 - Wave vs. Ovipositions

The final experiment was designed to determine how moth ovipositions, an extrinsic factor, interact with flower wave and flower positions, both intrinsic factors, to influence flower retention. Logistic regression of flower fate (abscised/retained) was used to examine the combined effects of the flower axil position, flower wave and the number of ovipositions. The best model (backward conditional) indicates that extrinsic ovipositions combine with intrinsic flower factors to influence retention (Table 3.4). To visualize the interplay of ovipositions, flower position/rank and flower wave a surface plot of the logistic regression equation is shown (Figure 3.5). Fruit retention is clearly influenced by the number of ovipositions but this is modified by whether the flower belongs to the first or second wave and also the relative linear position that the flower occupies on the inflorescence (Figure 3.5). Flowers from the bottom of the inflorescence have a lower probability of retention, which is similar to the pattern of retention we see under natural conditions. Finally, given high but identical numbers of ovipositions wave 2 flowers have higher probability of retention. This is not the pattern seen under open pollination or hand pollination of all flowers in the absence of moths (Chapter 2). Under these situations the wave 1 flowers have a higher probability of retention.

3.3 DISCUSSION

The results of this study on intrinsic and extrinsic influences on flower fate in the unbranched but multi-wave *Y. kanabensis* should be compared to a similar study on the branched *Y. filamentosa* (Huth and Pellmyr 1997). The present experimental study examined a number of intrinsic and extrinsic factors that affect yucca flower retention. Where possible, I attempted to separate factors that are normally interrelated, examining them separately or in small groups (Figure 3.1). I found that the interplay between the extrinsic moth-mediated effects of number of ovipositions and pollen loads and the intrinsic effects of flower position in the flowering sequence and flower wave is complicated and highly context dependent.

High pollen levels can influence selective retention of flowers but the effect is most pronounced in wave 1 flowers and in the absence of ovipositions made by deep moths (Figure 3.2 & 3.3a. & b.). Pollen levels can also interact with preferred and non-preferred flower waves compounding or lessening the wave differences depending upon the sequence of pollen presentation (Figure 3.3a. & b.). The positive effects of the high pollen levels studied can also be negated by the presence of deep moth ovipositions. Figure 3.4 shows this indirectly because total ovipositions was the only significant predictor of flower success even though two pollen levels were used in the experiment. Finally, moth ovipositions interact with both flower vertical position on the inflorescence and flower wave (Figure 3.5).

3.3.1 Ovipositions

The underlying assumption of this study was that surplus flowers in yuccas provide the plant with a large variation in flower quality from which a subset of the best flowers can be retained. I speculated that flower quality has two main components, those intrinsic to the plant, based on physiology and architecture, and those extrinsic to the plant but mediated by the moths. Moth mediated effects on flower quality are straightforward and simple to observe. First, deep moths lay eggs in the flowers and this not only causes short term physical damage to the ovary but long term damage in the form of constrictions and larval consumption of seeds. Hence, flower quality decreases as the number of ovipositions increases, making selection of flowers based in part on the number of ovipositions a prudent strategy

3.3.2 Pollen

Pollen deposition is also a moth-mediated extrinsic effect. There is vast variation in the amount of pollen flowers receive because moths may fail to pollinate, moths may attempt to pollinate but carry little or no pollen, or many moths may pollinate the same flower repeatedly (Addicott and Tyre 1995). For yuccas, the pollen continuum spans from flowers receiving no pollen to pollen far in excess of what is required. Fully fertilized yucca fruit have a cylindrical shape but partially fertilized fruit are tapered at the basal end in a way that would suggest that the pollen tubes growing down the stigma fertilize the first ovaries they encounter. Since it is rare to find naturally pollinated yucca fruit with a tapered shape indicating incompletely fertilized ovules, the minimum threshold for pollen defined by the number of ovules contained in a flower is met for at least the ~10% of flowers normally retained. However, the quality of fruit may still be improved above full fertilization if excess pollen grains compete for ovules, an effect which has been demonstrated in many plants (Schlichting *et al.* 1990).

3.3.3 Pollen and Ovipositions Combined

The present study focused on two pollen loads both of which are in excess of the minimum required. In the absence of moth ovipositions there is evidence to indicate that this is affects flower retention. However the lack of interaction between pollen load and ovipositions (Figure 3.4) in the present study suggests that in the range of pollen that I used the improvement to fruit quality above the pollen grains required for full fertilization is less important than the number of ovipositions to flower retention. This ability for the plant to discriminate amongst flowers based on pollen load is relevant because it could reinforce the behavior of moths that are efficient pollinators and select against the behavior of moths that fail to pollinate or deliver few grains. However, it would be dangerous from a plant fitness perspective to select flowers solely on the basis of pollen because flowers visited by many efficiently pollinating moths could also carry very high egg loads although the correlation between number of eggs and amount of pollen is weak (JFA unpublished data).

3.3.3.4 Intrinsic Effects

The second component of flower quality is intrinsic and is related to flower position on the inflorescence relative to the source of resources as well as when the flower opens in the blooming sequence. Although intrinsic differences are widely believed to exist and have been documented for other plants, only two studies have considered yuccas in this context. The first study reported that fruit from the top of *Y. whipplei* inflorescences had lighter weight seeds than fruit from the bottom (Aker 1982), but the second study (see Chapter 2) was unable to find any measurable differences from fruit from different positions on the inflorescence. Nevertheless, inherent differences in the potential of flowers to become fruit exist even if they do not have a basis in measured fruit qualities. This is because flowers located near the base of the plant are physically able to preempt resources that could be used by flowers higher up the inflorescence.

The explanation for the overall differential preference for the 1st flowering wave in Experiment 3 is unknown. This wave may have an advantage of timing, or position and there are in fact dry weight differences of flowers from different flower waves (see Chapter 2). These differences in size and position may make some flowers more aggressive sinks for resources. Since little is known about the behavior of individual moths, it is unclear how the plant's flower wave preference may affect moths. If moths do not show selection of flowers from a particular wave, the presence of other flower waves may act to dilute the utilization of the wave 1 flowers as oviposition sites or they may also function to add pollen to the system. I have also documented a positional effect along the inflorescence in addition to the wave effect, and these two intrinsic features interact with pollen or ovipositions to modify flower retention. These intrinsic effects are most interesting because they create a window of opportunity for non-mutualistic moth behavior that might otherwise be selected against. For example a female that was inefficient at, or neglectful of transferring pollen might still be successful compared to a very efficient female if she transferred an adequate amount of pollen to a flower that was inherently more likely to become a fruit.

3.3.3.5 Combined Intrinsic and Extrinsic Features

The interaction between pollen loads, ovipositions and intrinsic flower features is critical because none of these features offer the plant a foolproof way of assessing flower quality. Flowers selected strictly on the basis of low numbers of ovipositions might have insufficient pollination and be unable to compete for maternal resources effectively because of relative position on the inflorescence. Flowers selected solely on the basis of excellent pollen might have excessive numbers of ovipositions and selecting flowers on the basis of wave or position might allow the proliferation on non-mutualistic moth tendencies. The interplay between these factors allows the plant to respond to a variety of situations in an adaptive way. It also provides clues as to how the yuccas respond to the shallow moths that do not trigger abscission based on ovipositions. This moth only has a short ovipositor and lays its eggs superficially at the surface of the pistil. After hatching, the 1st instar larvae then burrow through the wall of the immature fruit into the seeds. This species does not cause fruit constrictions and does not trigger selective abscission of flowers based on the number of ovipositions (Wilson and Addicott 1998). The shallow moths appear to have an advantage over the deep moths with respect to triggering selective abscission based on ovipositions. However, the shallows have not excluded the deeps but instead coexist at several sites suggesting that the shallows must be regulated through another process. This could be based on a density process between moths themselves or perhaps through pollen loads or the intrinsic flower effects.

Combined results from the five experiments indicate that the interaction between the plant and the pollinator is dynamic, making it difficult for a defined set of ‘rules’ for plant and moth actions to be discovered. The factors that influence which flowers successfully become fruit are multifaceted and highly context dependent. This may in part offer an explanation as to why we do not see selection for behavioral adaptations on the part of moths which would allow them to avoid flowers likely to abscise (Wilson and Addicott 1998).

3.4 CONCLUSIONS

Surplus flowers in yuccas create an opportunity for selective retention of flowers of high quality. Quality has both intrinsic and extrinsic components and they interact creating a situation where flower quality is highly context dependent. Through selective abscission based on many aspects of flower quality yuccas regulate costs and benefits in the face of a mutualistic partner with non-mutualistic tendencies.

Table 3.1 Logistic regression analysis (backward conditional model fitting) of flower fate data for Experiment 3a. Pollen Quantity-Waves Combined where high pollen was delivered first followed by low pollen. Both flower wave and pollen load are significant predictor variables of flower success. Overall model Chi-Square=17.805, df=2, p=0.0001

Term	**Coefficient**	***df***	**Significance**
Flower Wave	1.0700	1	p=0.0010
Pollen	0.8453	1	p=0.0091
Constant	−1.349		

Table 3.2. Logistic regression analysis (backward conditional model fitting) of flower fate data for Experiment 3b. Pollen Quantity-Waves Combined with low pollen first followed by the high pollen treatment. The flower wave by pollen interaction is the only significant predictor variable of flower success. Overall model Chi-Square=38.446, df=1, p<0.0001

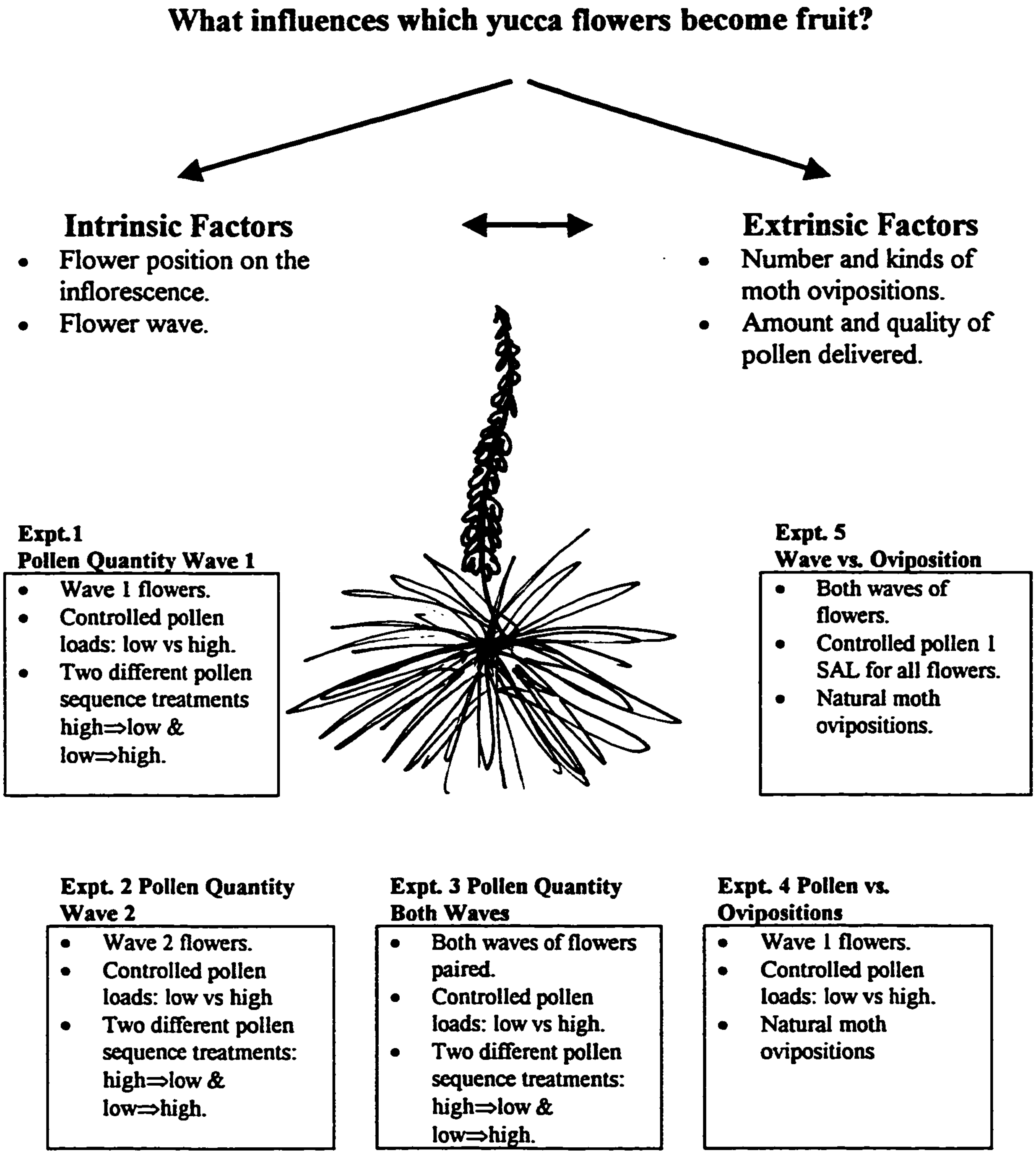
Term	**Coefficient**	***df***	**Significance**
Flower Wave X Pollen Interaction	2.4751	1	p<0.0001
Constant	–0.6042		

Table 3.3 Logistic regression analysis (backward conditional model fitting) of flower fate data for the Experiment 4. Pollen Load vs. Ovipositions. The total number of ovipositions a flower receives is the only significant predictor variable of flower success. Overall Model Chi-Square=35.311, df=1, p<0.0001

Term	**Coefficient**	***df***	**Significance**
Total Ovipositions	-.1460	1	p<0.0001
Constant	-.1969		

Table 3.4. Logistic regression analysis (backward elimination) was used to examine the combined effects of the flower axil position, flower wave and the number of ovipositions on flower fate. Significant predictor variables of flower success are shown below. Overall Model Chi-Square=68.507, df=1, p<0.0001.

Term	**Coefficient**	***df***	**Significance**
adjusted axil X flower wave	.2420	1	p=0.0002
total ovipositions	-0.0881	1	p<0.0001
flower wave X total ovipositions	-.1136	1	p=0.0019
Constant	.2759		



What influences which yucca flowers become fruit?

Intrinsic Factors

- Flower position on the inflorescence.
- Flower wave.

Extrinsic Factors

- Number and kinds of moth ovipositions.
- Amount and quality of pollen delivered.

Expt.1
Pollen Quantity Wave 1

- Wave 1 flowers.
- Controlled pollen loads: low vs high.
- Two different pollen sequence treatments high⇒low & low⇒high.

Expt. 5
Wave vs. Oviposition

- Both waves of flowers.
- Controlled pollen 1 SAL for all flowers.
- Natural moth ovipositions.

Expt. 2 Pollen Quantity Wave 2

- Wave 2 flowers.
- Controlled pollen loads: low vs high
- Two different pollen sequence treatments: high⇒low & low⇒high.

Expt. 3 Pollen Quantity Both Waves

- Both waves of flowers paired.
- Controlled pollen loads: low vs high.
- Two different pollen sequence treatments: high⇒low & low⇒high.

Expt. 4 Pollen vs. Ovipositions

- Wave 1 flowers.
- Controlled pollen loads: low vs high.
- Natural moth ovipositions

Figure 3.1 Overview of the experiments performed to investigate the effects of intrinsic and extrinsic flower variation in the production of yucca fruit.

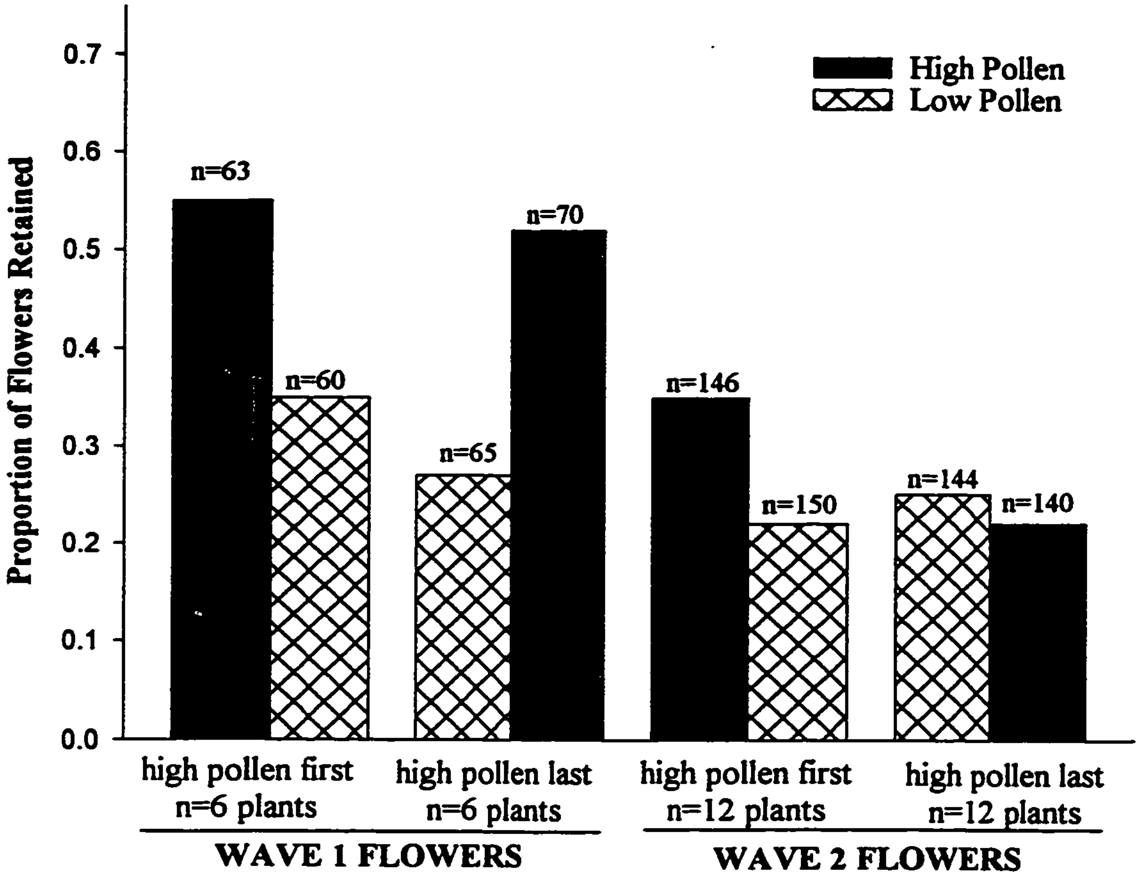


Figure 3.2 Proportion of fruit resulting from two different pollen levels, high and low (1 SAL and 1/4 SAL respectively), from 4 separate experiments. Two experiments utilized wave 1 flowers only (first 2 bar clusters) and two utilized wave 2 flowers only (last 2 bar clusters). Within each wave experiment there were two possible pollen delivery treatments: high pollen followed by low pollen and low pollen followed by high pollen.

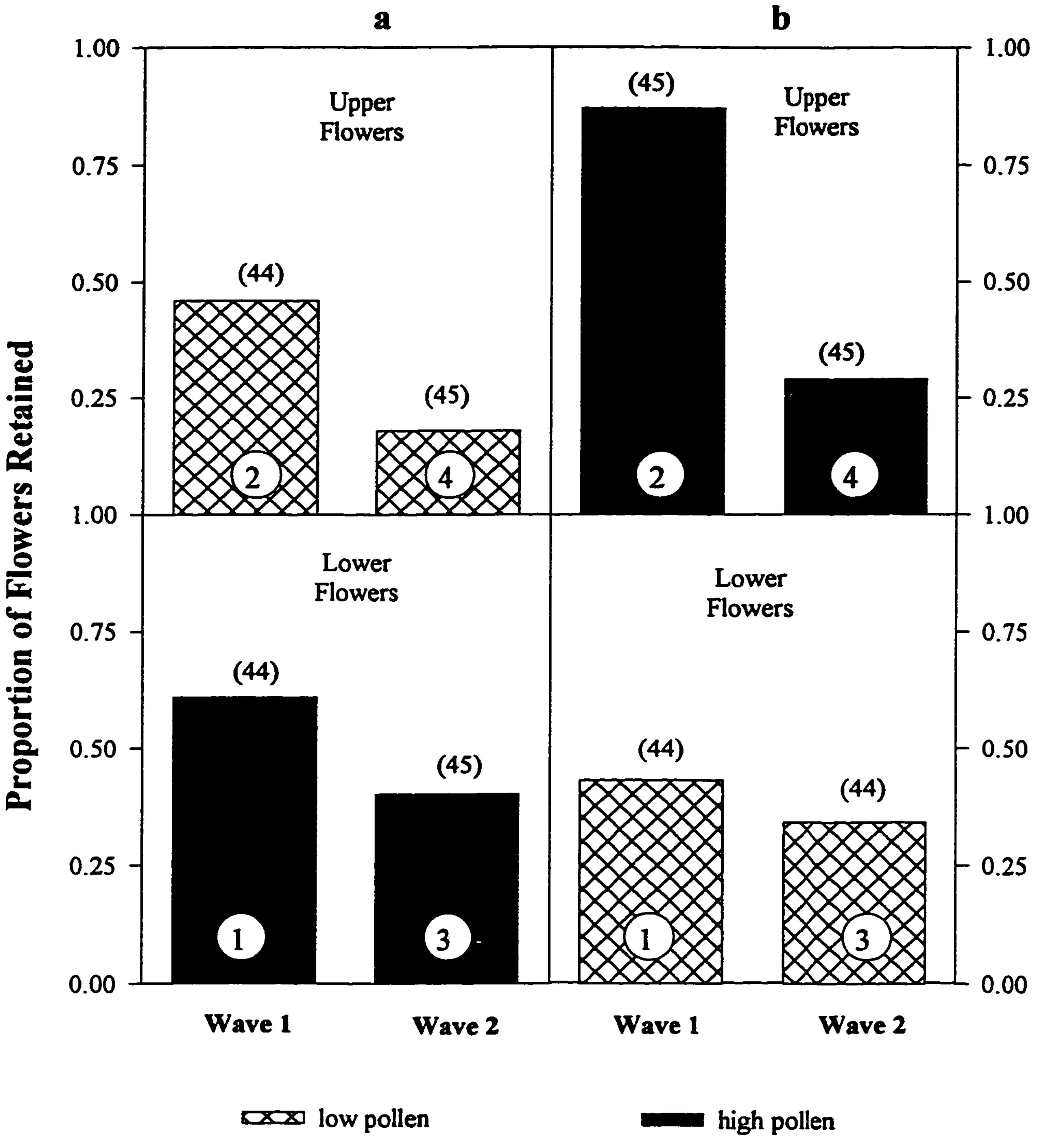


Figure 3.3 a. & b. Proportion of flowers retained after receiving either a high or low pollen load. The lower panel shows the first 5 axils with paired wave 1 flowers (left) and wave 2 flowers (right) receiving high pollen (a.) or low pollen (b.). The upper panel shows the last 5 axils with wave 1 flowers (left) and wave 2 flowers (right) receiving either low pollen (a.) or high pollen (b.). Numbers within bars show flowering sequence 1=early wave 1, 2=late wave 1, 3=early wave 2, 4=late wave 2. Flowers labelled 2nd and 3rd can overlap in flowering.

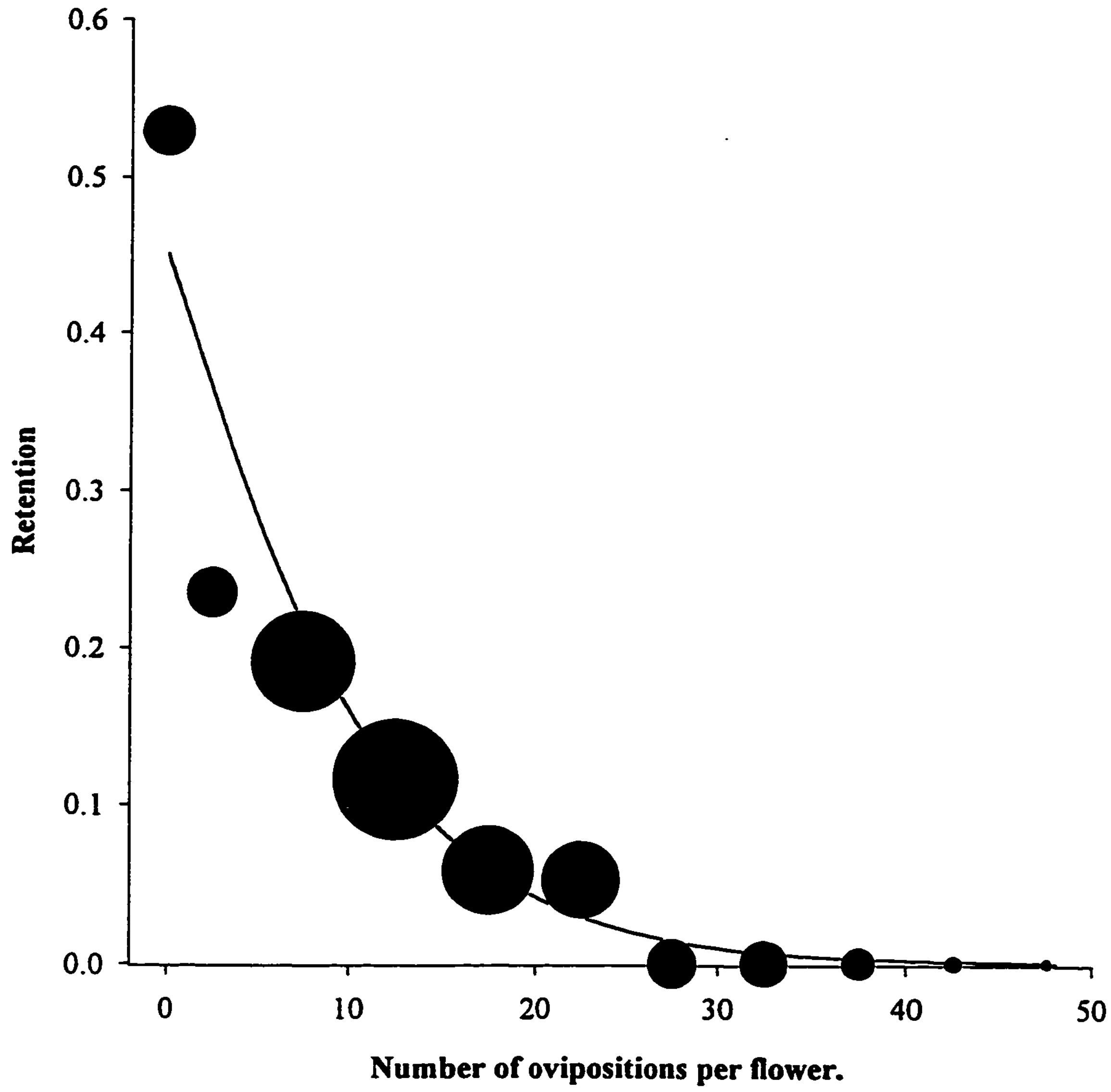


Figure 3.4 Probability of flower retention (generated from logistic regression of fruit presence/absence) as a function of number of ovipositions a flower received. Sample sizes are proportional to the area of the circles (n=324 flowers).

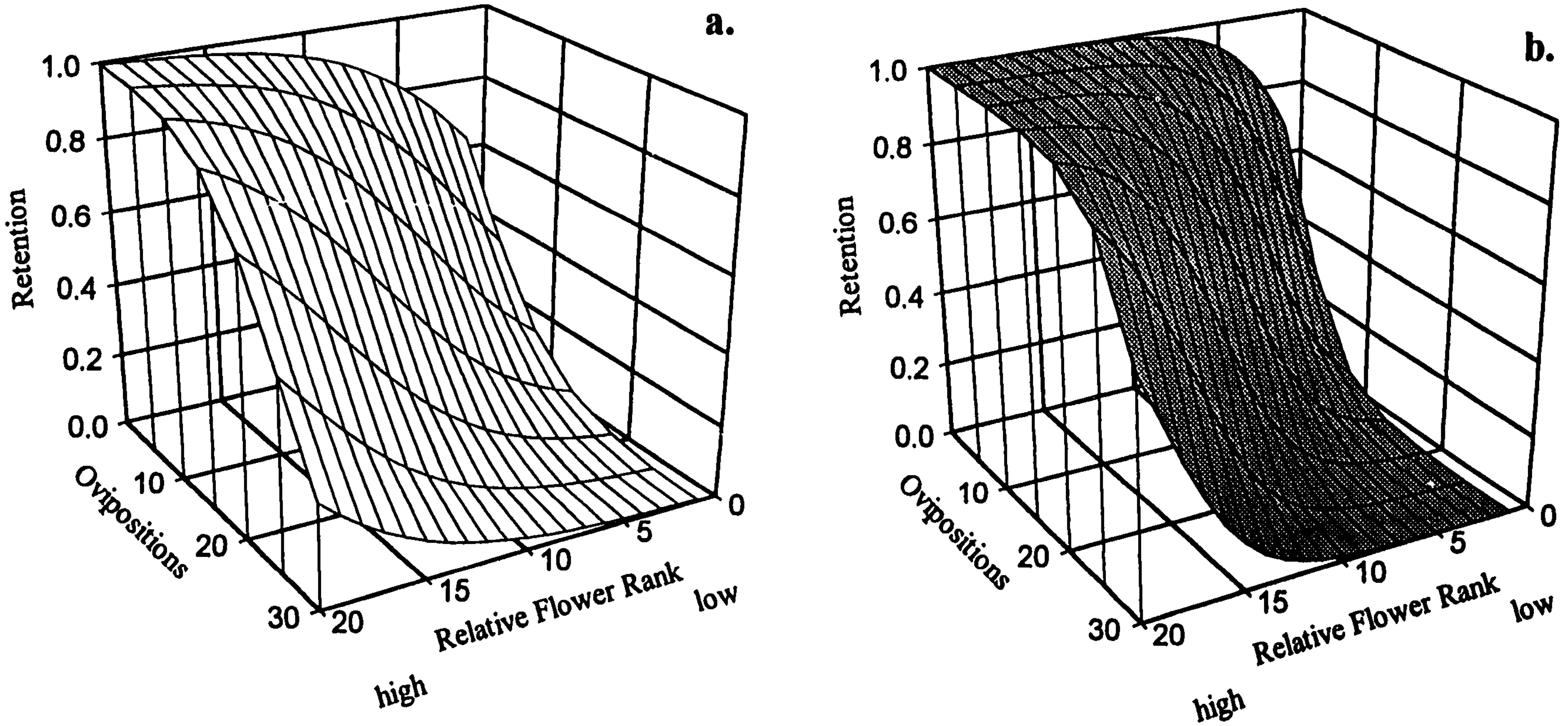


Figure 3.5. Surface plots showing results from logistic regression analysis. Regression was calculated from actual data using backward elimination test procedures. Number of ovipositions was the strongest predictor of flower flower fate and flower wave interacted both with number of ovipositions and adjusted axil. (a) function of wave 1 and (b) wave 2 flowers are shown separately.

3.5 LITERATURE CITED

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. Oikos **81**:119-129.

Addicott, J. F., and A. J. Tyre. 1995. Cheating in an obligate mutualism: how often do yucca moths benefit yuccas? Oikos **72**:382-394.

Aker, C. L. 1982. Regulation of flower, fruit, and seed production by a monocarpic perennial, *Yucca whipplei*. Journal of Ecology **70**:357-372.

Aker, C. L. and D. Udovic. 1981. Oviposition and pollination behavior of the yucca moth, *Tegeticula maculata* (Lepidoptera: Prodoxidae), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). Oecologia **49**:96-101.

Becerra, J. X. and D. G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormia tenax* (Agaraceae): a second action of self-incompatibility at the whole flower level? Evolution **46**:458-469.

Brunet, J. 1996. Male reproductive success and variation in fruit set in *Aquilegia caerulea* (Ranunculaceae). Ecology 77:2458-2471.

Cruzan, M. B. 1990. Pollen-pollen and pollen-style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). American Journal of Botany 77:116-122.

Dodd, R. J. and Y. Linhart. 1994. Reproductive consequences of interactions between *Yucca glauca* (Agavaceae) and *Tegeticula yuccasella* (Lepidoptera) in Colorado. American Journal of Botany **81**:815-825.

Ehrlen, J. 1993. Ultimate functions of non-fruiting flowers of *Lathyrus vernus*. Oikos **68**:45-52.

Fuller, O. S. 1990. Factors affecting the balance of co-operation and conflict between the yucca moth, *Tegeticula yuccasella* and its mutualist, *Yucca glauca*. PhD Thesis, University of New Mexico, Albuquerque, New Mexico, USA.

Guitian, J. 1994. Selective fruit abortion in *Prunus mahaleb* (Rosaceae). American Journal of Botany **81**:1555-15558.

Guitian, J., P. Guitian, and L. Navarro. 1996. Fruit set, fruit reduction, and fruiting strategy in *Cornus sanguinea* (Cornaceae). American Journal of Botany **83**:744:748.

Herrera, J. 1991. Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Laminaceae). American Journal of Botany **78**:789-794.

Holtsford, T. P. 1985. Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. American Journal of Botany **72**:1687-1694.

Huth, C. and O. Pellmyr. 1997. Non-random fruit retention in Yucca *filamentosa*: consequences for an obligate mutualism. Oikos **78**:576-584.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1993. Pollination ecology of *Yucca elata*. An experimental study of a mutualistic association. Oecologia **93**:512-517.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1994. Fruit abortion in *Yucca elata* and its implications for the mutualistic association with yucca moths. Oikos **69**:207-216.

Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature **372**:257-260.

Pellmyr, O., J. N. Thompson, J. M. Brown and R. G. Harrison. 1996. Evolution of pollination and mutualism in the yucca moth lineage. The American Naturalist **148**:827-847.

Pellmyr, O., L. K. Massey, J. L. Hamrick and M. A. Feist. 1997. Genetic consequences of specialization: yucca moth behavior and self-pollination in yuccas. Oecologia **109**:273-278.

Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. Trends in Ecology and Evolution 7:10-15.

Riley, C.V. 1872. The fertilization of the yucca plant by *Pronuba yuccasella*. Canadian Entomologist **4**:182.

Schlichting, C. D., A. G. Stephenson, and L. E. Small. 1990. Pollen loads and progeny vigor in *Curcurbita pepo*: the next generation. Evolution **44**:1358-1372.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253-279.

Stephenson, A. G., B. Devlin, and J. B. Horton. 1988. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Curcurbita pepo* (Zucchini squash). Annals of Botany **62**:653-661.

Sutherland, S. 1987. Why hermaphroditic plants produce many more flowers than fruits: experimental tests with *Agave mckelveyana*. Evolution **41**:750-759.

Tyre, A. J. and J. F. Addicott. 1993. Facultative non-mutualistic behavior by an "Obligate" mutualist: "Cheating" by yucca moths. Oecologia **94**:173-175.

Wilson, R. D., and J. F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos **81**:109-118.

Winsor, J. A., L. E. Davis, and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Curcurbita pepo*. The American Naturalist **129**:643-656.

Wyatt, R. 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. American Journal of Botany **69**:585-594.

Young, H. J. and T. P. Young. 1992. Alternative outcomes of natural and experimental high pollen loads. Ecology **73**:639:647.

Ziv, Y. and J. L. Bronstein. 1996. Infertile seeds of *Yucca schottii*: a beneficial role for the plant in the yucca-yucca moth mutualism? Evolutionary Ecology **10**:63-76.

Zar, J. H. 1996. Biostatistical analysis 3rd Ed. Prentice Hall, New Jersey.

Chapter 4

Thesis Discussion

4.0 DISCUSSION

The obligate pollination/seed predation mutualism between yuccas and yucca moths is a balanced conflict of fitness interests (Fuller 1990). The yucca moth attempts to maximize its fitness by laying as many eggs as possible in as many yucca flowers as possible (Pellmyr and Huth 1994). This in turn reduces yucca fitness because of the seed loss that results from larval feeding. In spite of the conflict, neither partner can 'defect' from the mutualism because both partners require the association to reproduce sexually (Axelrod and Hamilton 1981, Addicott and Tyre 1995). The inability to 'defect' suggests that more strategic solutions are required to stabilize the conflict in this mutualism.

The intensity of the conflict between the partners varies because the costs and benefits to the partners may fluctuate from season to season. In some years, yucca moths may behave more like pollinating parasites by leaving yuccas few or no seeds at the end of the season. In other years, larval feeding may be so light that the effect of the moth is negligible. Conditional outcomes in this, and other mutualisms, are influenced by both abiotic conditions (e.g. weather or nutrient conditions) and biotic conditions (e.g. partner density or presence/absence of other species). Further, in some mutualisms, partners are capable of regulating the interaction in a way that stabilizes them and limits partners from taking advantage of each other. Regulation of partners can be achieved by altering partner behavior, physiology or density (See Chapter 1).

During the early phase of research in the yucca/yucca moth mutualism it was thought that yucca moths 'restrained' themselves in the number of eggs laid (Riley 1892, Trelease 1893, Aker and Udovic 1981, James *et al.* 1993). Later it became clear that moths do not restrain themselves or avoid flowers that other moths have visited thus compounding the problem of flower over-utilization (Addicott and Tyre 1995, Wilson and Addicott 1998). In addition yucca moths may offer poor quality pollination service either by failing to pollinate (Tyre and Addicott 1993) or by delivering self-pollen (Pellmyr *et al.* 1997). However yuccas are not passive 'victims' to moth exploitation. Several recent studies show that yuccas are sensitive to the number of ovipositions that individual flowers receive and use selective abscission to remove flowers with high numbers of ovipositions

(Addicott 1998, Pellmyr and Huth 1994, Richter 1995, Richter and Weis 1995). This is an effective strategy for a resource-limited plant that produces far more flowers than it will mature. By regulating the cost of the association through a direct manipulation of the moth larval feeding density that the plant tolerates, yuccas stabilize the mutualism. However, it is unlikely that the number of ovipositions is the only factor that yuccas respond to. Some studies have indicated that intrinsic factors affecting flower retention or fruit features exist (Aker 1982, Ziv and Bronstein 1996, Huth and Pellmyr 1997) and may indirectly contribute to partner regulation. The main focus of the present study was to refine our understanding of partner regulation in the yucca/yucca moth mutualism by examining the role of fruit set patterns and function of surplus flowers in *Yucca kanabensis* McKelvey.

4.0.1 The role of fruit set patterns

Non-random fruit retention patterns have been described for several species of yucca (Aker and Udovic 1981, Aker 1982, Powell and Mackie 1966, James *et al.* 1994, Huth and Pellmyr 1997). One study in particular indicated that fruit set patterns might be part of the regulatory processes, because fruit from different areas of the inflorescence had seeds with different weights (Aker 1982). Since yuccas exchange seeds for pollination service, anything that could increase the number of seeds available for dispersal would decrease the cost of the association to the yucca. I speculated that this could occur if fruit set patterns allowed yuccas to produce larger fruit or decreased the amount of time larvae spent feeding within fruit. This would occur if yuccas selectively matured fruit that were going to be larger, as was seen by Aker (1982). Larger fruit would either have more seeds overall, the same number of larger seeds or both; and providing the average number of larvae per fruit was maintained, this would presumably benefit yuccas.

In Chapter 2 I investigated if fruit retention patterns in *Y. kanabensis* allowed for retention of higher quality fruit. First I determined the intrinsic pattern of fruit retention in the absence of moth oviposition and with hand pollination. Results showed that there was strong selection for certain flowers based on spatial and temporal arrangement. There was selection both for flowers from the first wave of flowering (Table 2.1, Table

2.2) and proximal flowers (Figure 2.2). Neither of these patterns was different for the two levels of pollination that I investigated (Figure 2.2). Collections of yucca flowers provide some clues to this preference. Flowers at the same axil position but from different flowering waves are not the same weight. The wave 1 flowers weigh more than the flowering waves that follow (Figure 2.3). In addition, when all waves of flowers are collected from a consecutive sequence of flower axils, there is a decrease in flower weight, both within and between waves, toward the distal end of the inflorescence (Figure 2.4). There is a coincidental preference in retention for flowers that weigh more.

Once the intrinsic patterns of fruit retention were determined I questioned whether they contributed to the regulation of this mutualism. I tried tosimulate preferred and non-preferred fruit set patterns under controlled conditions to determine if the patterns affected fruit or larval qualities related to fitness. I was unable to detect any significant difference in fruit features such as size, weight or seed number (Figure 2.5, Table 2.6). This failure to detect a difference may have three explanations: first there may be no variation in fruit quality from different areas of the inflorescence in *Y. kanabensis;* second, my ability to detect these differences was low (power ranged between 15-22%) and third, there may be greater variation in the differential contribution of fruit from different flowering waves which was not measurable in my experiments.

In addition to examining fruit differences I also examined larval differences such as number of larvae per fruit, number of seeds eaten, length of time to emergence and larval size. I was unable to find any trends in larval features that would support the hypothesis that fruit set patterns might increase yucca fitness by decreasing costs incurred by larval feeding (Figure 2.7, Figure 2.9). Do fruit set patterns offer insight into the regulation of yucca moths by yuccas? The current study provides no evidence to indicate that this occurs but a new experiment combining what was learned from the present study may answer this question more effectively.

I next examined the extrinsic retention patterns of open pollinated plants and compared this to my intrinsic pattern. The open pollinated plants differed in two important ways.

First, they were exposed to moth ovipositions, which are known to influence flower success. Second, the quality and quantity of pollen delivered were variable. The intrinsic and extrinsic patterns were both non-random but different from each other. Both intrinsic and extrinsic patterns show active discrimination against flowers from the distal third of the inflorescence (Figure 2.2). However, in the extrinsic pattern the middle group of flowers contributes the most to fruit set while the intrinsic pattern favors the proximal third (Figure 2.2). Some aspects of pollinator behavior may explain this shift. Yucca moths tend to favor the freshest distal flowers each night and lay more eggs in distal fresh flowers (Wilson and Addicott 1998). The extrinsic pattern seen may then represent the 'compromise' between the flowers the plant prefers to mature (if lightly visited) and the ones the moths prefer to visit.

4.0.2 The role of surplus flowers and intrinsic vs. extrinsic factors

The second part of this study further examined the role that surplus flowers play in the regulation of this mutualism. It is widely agreed that for many plants surplus flowers allow selectivity for offspring of higher quality (reviewed in Stephenson 1981). For plants, flower and subsequent fruit quality may be affected by the source and quantity of pollen received (Winsor *et al.* 1987, Stephenson *et al.* 1988, Cruzan 1990, Schlichting *et al.* 1990, Becerra and Lloyd 1992, Young and Young 1992), the position of the flower in the opening sequence or the actual position that the flower occupies in the inflorescence (Wyatt 1982, Holtsford 1985, Sutherland 1987, Stephensen *et al.* 1988, Guitian 1994, Diggle 1995). In yuccas these intrinsic and extrinsic factors also operate and are combined with the obvious negative effect that ovipositions have on flower quality. I attempted to learn how these factors contribute to the success or failure of surplus flowers and how this may ultimately affect this mutualism. I separately investigated how flowers from different waves respond to variation in pollen loads demonstrating that both flower waves are responsive to variation in pollen loads, usually preferentially retaining flowers with more pollen (Figure 3.2). I then examined the interplay between high and low pollen loads and preferred and non-preferred waves of flowers, and how the presence of flowers from different waves affects which ones become fruit. High pollen loads and preferred flower waves can compound flower success but preferred pollen loads delivered to non-preferred flower waves can change which flowers tend to become fruit (Figure 3.3). When ovipositions are added back into the system the pollen levels that I investigated no longer had an influence when only 1 wave of flowers was used (Figure 3.4). However in a more complex experiment using two waves of flowers, controlled pollen and variable ovipositions the effects of ovipositions and flower position on the inflorescence as well as flower wave all contribute to the explanation of which flowers become fruit (Figure 3.5). Do surplus flowers provide yuccas with an ability to selectively retain flowers of higher quality? The present study demonstrates that under certain conditions yuccas can respond to variation in pollen levels, flower positions and moth ovipositions in a way that would suggest that flower quality is improved.

4.1 CONCLUSION

Yucca kanabensis flowers vary in their ability to contribute to fruit set. If all flowers are pollinated there is selection for flowers that open early and are from the first wave of flowering. I was unable to show that this preference ultimately manifests itself in differential fruit or larval quality that benefits yuccas. In addition to the intrinsic features that affect flower retention I investigated extrinsic features and their interactions. When all of these results are combined it becomes clear that the factors which affect fruit retention are complicated. Intrinsic fruit retention patterns can be changed by interacting with variation in moth ovipositions and pollen delivery.

Initially it seems surprising that a simple set of 'rules' defining which flowers become fruit has not been discovered. However, selecting flowers only on the basis of one of the following: ovipositions, pollen, flower wave, axil position or opening date, does not offer a foolproof way for selecting which flowers are of the highest quality. Having a flexible set of multifaceted 'rules' allows yuccas to respond successfully to variation caused by several species of pollinators (deeps and shallows) under a variety of conditions. Moths do not appear to respond strongly to obvious information about the pollination or oviposition status of flowers they visit (Wilson and Addicott 1998). Thus it seems even less likely that they can assess less obvious information such as what flower wave they are visiting, relative flower opening date or relative flower position on the inflorescence.

This inability to assess even simple information about flowers also makes it difficult for the moths to develop behaviors that reliably allow them to improve their fitness. The same behavior that results in a reward in one flower might result in punishment in the next.

4.2 LITERATURE CITED

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. Oikos **81**:119-129.

Addicott, J. F., and A. J. Tyre. 1995. Cheating in an obligate mutualism: how often do yucca moths benefit yuccas? Oikos **72**:382-394.

Aker, C. L. 1982. Regulation of flower, fruit, and seed production by a monocarpic perennial, *Yucca whipplei*. Journal of Ecology **70**:357-372.

Aker, C. L. and D. Udovic. 1981. Oviposition and pollination behavior of the yucca moth, *Tegeticula maculata* (Lepidoptera:Prodoxidae), and its relation to the reproductive biology of *Yucca whipplei* (Agavaceae). Oecologia **49**:96-101.

Axelrod, R. and D.W. Hamilton. 1981. The evolution of cooperation. Science **211**:1390-1396.

Becerra, J. X. and D. G. Lloyd. 1992. Competition-dependent abscission of self-pollinated flowers of *Phormia tenax* (Agaraceae): a second action of self-incompatibility at the whole flower level? Evolution **46**:458-469.

Cruzan, M. B. 1990. Pollen-pollen and pollen-style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). American Journal of Botany 77:116-122.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. Annual Review of Ecology and Systematics **26**:531-552.

Fuller, O. S. 1990. Factors affecting the balance of co-operation and conflict between the yucca moth, *Tegeticula yuccasella* and its mutualist, *Yucca glauca*. PhD Thesis, University of New Mexico, Albuquerque, New Mexico, USA.

Guitian, J. 1994. Selective fruit abortion in *Prunus mahaleb* (Rosaceae). American Journal of Botany **81**:1555-15558.

Holtsford, T. P. 1985. Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. American Journal of Botany **72**:1687-1694.

Huth, C. and O. Pellmyr. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. Oikos **78**:576-584.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1993. Pollination ecology of *Yucca elata*. An experimental study of a mutualistic association. Oecologia **93**:512-517.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes and W. G. Whitford. 1994. Fruit abortion in *Yucca elata* and its implication for the mutualistic association with yucca moths. Oikos **69**:207-216.

Pellmyr, O. and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature **372**:257-260.

Pellmyr, O., L. K. Massey, J. L. Hamrick and M. A. Feist. 1997. Genetic consequences of specialization: yucca moth behavior and self-pollination in yuccas. Oecologia **109**:273-278.

Powell, J. A. and R. A. Mackie. 1966. Biological interrelationships of moths and *Yucca whipplei* (Lepidoptera: Gelechiidae, Blastobasidae, Prodoxidae). University of California Publications in Entomology **42**:1-59

Richter, K. S. 1995. The pollination of *Yucca whipplei* by *Tegeticula maculata*: the benefits and costs of an obligate mutualism. Ph.D. Dissertation. University of California, Irvine. USA.

Richter, K. S. and A. E. Weis. 1995. Differential abortion in the yucca. Nature **376**:557-558.

Riley, C. V. 1892. The yucca moth and yucca pollination. Annual Report of the

Missouri Botanic Garden **3**:99-159.

Schlichting, C. D., A. G. Stephenson, and L. E. Small. 1990. Pollen loads and progeny vigor in *Curcurbita pepo*: the next generation. Evolution **44**:1358-1372.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253-279.

Stephenson, A. G., B. Devlin, and J. B. Horton. 1988. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Curcurbita pepo* (Zucchini squash). Annals of Botany **62**:653-661.

Sutherland, S. 1987. Why hermaphroditic plants produce many more flowers than fruits: experimental tests with *Agave mckelveyana*. Evolution **41**:750-759.

Trelease, W. 1893. Further studies of yuccas and their pollination. Annual Report of the Missouri Botanic Garden **4**:181-226.

Tyre, A. J. and J. F. Addicott. 1993. Facultative non-mutualistic behavior by an "Obligate" mutualist: "Cheating" by yucca moths. Oecologia **94**:173-175.

Wilson R. D. and J.F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos **81**:109-118.

Winsor, J. A., L. E. Davis, and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Curcurbita pepo*. The American Naturalist **129**:643-656.

Wyatt, R. 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. American Journal of Botany **69**:585-594.

Young, H. J. and T. P. Young. 1992. Alternative outcomes of natural and experimental high pollen loads. Ecology **73**:639:647.

Ziv, Y. and J. L. Bronstein. 1996. Infertile seeds of *Yucca schottii*: a beneficial role for the plant in the yucca-yuccca moth mutualism? Evolutionary Ecology **10**:63-76.

IMAGE EVALUATION
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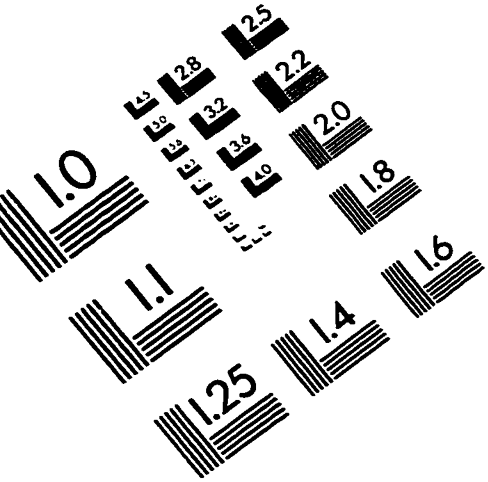

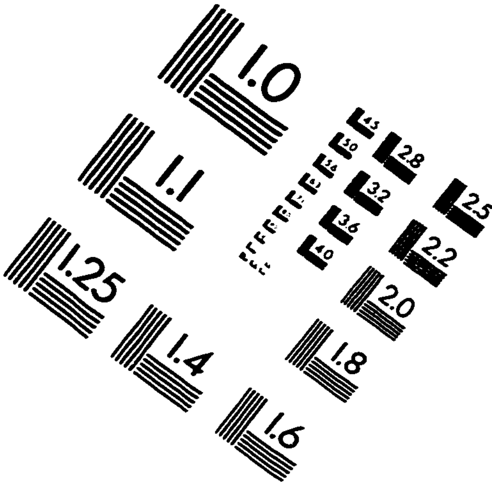

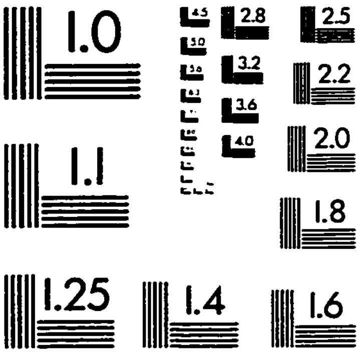

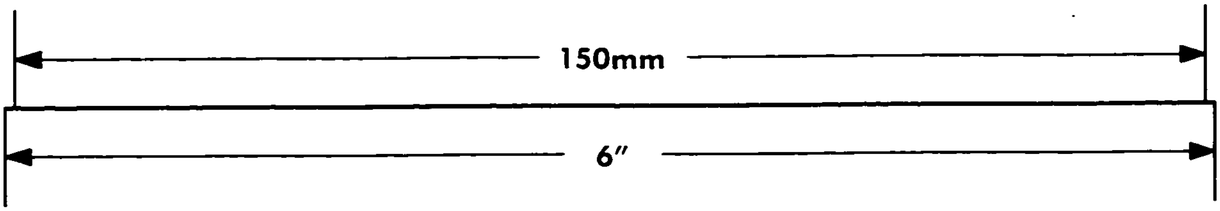

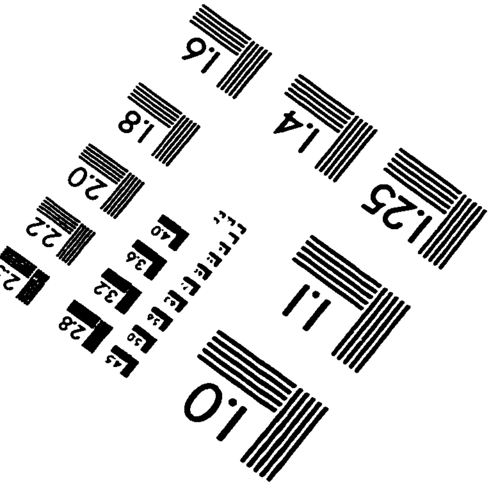

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