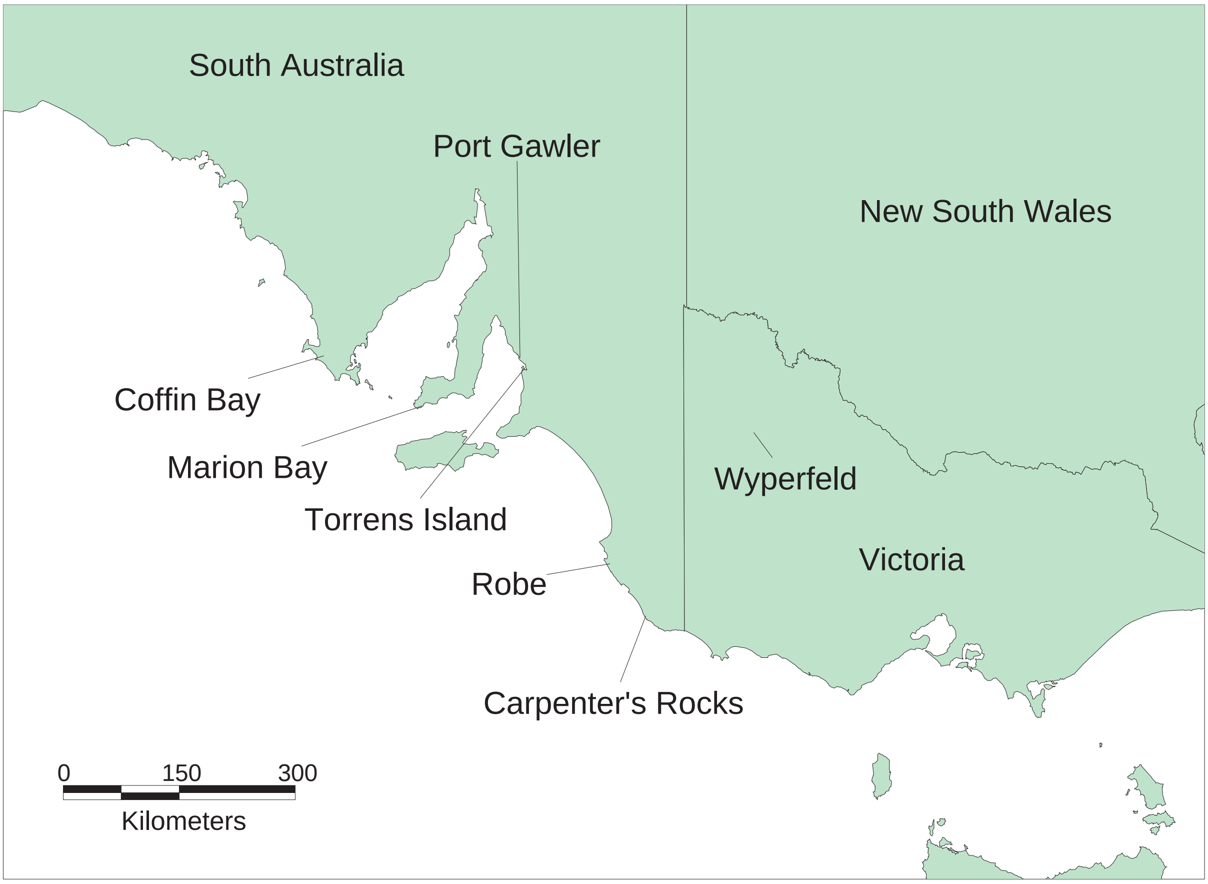
Seed biology of Australian euphorbs:



Areas used for collection of seed of *Adriana* species

Final Report for the Australian Flora Foundation Inc

by

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29 March 1998

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Summary

Within the Euphorbiaceae, many species have seeds that bear an appendage called a caruncle, although carunculate seeds have a sporadic taxonomic distribution, with some euphorb genera containing both carunculate and ecarunculate species (Webster 1994). The euphorb caruncle is often assumed to function as an elaiosome, that is, as an appendage that facilitates seed dispersal by ants, or myrmecochory. This assumption has received very little quantitative or experimental verification.

Our work on the euphorb *Adriana* concentrated on determining whether or not carunculate seeds are dispersed by ants and whether or not the caruncle is an attractant to seed-dispersing ants. Together, the field and laboratory choice trials indicated that the caruncle is attractive to ants and that carunculate seeds are particularly attractive to omnivorous ants of the genera *Rhytidoponera* and *Iridomyrmex*.

Since elaiosomes have been hypothesised to have evolved so as to resemble arthropods in their fatty acid composition, we compared the fatty acid compositions of blowflies and *Adriana* seed and caruncles. Blowflies resembled *Adriana* caruncles in having relatively low concentrations (10%) of linoleic acid and intermediate concentrations (18%) of palmitic acid, in contrast to *Adriana* seeds which have relatively high concentrations (62%) of linoleic acid and relatively low concentrations (5%) of palmitic acid.

The caruncle:seed mass ratio has been shown to be an important cue eliciting diaspore removal in other studies Hence they were analysed in populations of *Adriana quadripartite*. Analysis of diaspore and caruncle weights revealed that while there is significant variation in seed weight, the relationship between seed weight and caruncle weight does not vary among populations, and hence the caruncle:seed mass ratio does not vary significantly among the sampled populations.

Introduction

This report describes the results of our researches into the nature and function of the caruncle on seeds of plants in the endemic euphorb genus *Adriana*. Within the Euphorbiaceae, many species have seeds that bear an appendage called a caruncle, although carunculate seeds have a sporadic taxonomic distribution, with some euphorb genera containing both carunculate and ecarunculate species (Webster 1994).

The euphorb caruncle is often assumed to function as an elaiosome, that is, as an appendage that facilitates seed dispersal by ants, or myrmecochory. This assumption has received very little quantitative or experimental verification. Our work on *Adriana* has concentrated on determining whether or not carunculate seeds are dispersed by ants and whether or not the caruncle is an attractant to seed-dispersing ants. We have also investigated the chemical composition of caruncles of *Adriana* and of *Ricinus* and we have compared our results with those from other chemical analyses of elaiosome constituents. Finally, we have determined the extent of variation in the relative size of the caruncle within and between populations of *Adriana*.

Does the caruncle serve as an elaiosome to attract seed-dispersing ants?

This question was answered using a series of field and laboratory observations and trials.

Field trials:

Trials were conducted in several different populations of *Adriana quadripartita* in South Australia. Petri dishes containing equal numbers of intact diaspores, seeds without caruncles and caruncles were placed on the ground near *Adriana* plants. The identity of ants removing these items was recorded and the numbers of diaspores, seeds and caruncles remaining in the dishes at the end of the trial were also recorded. Ants in the genera *Iridomyrmex*, *Melophorus* and *Rhytidoponera* were observed removing seeds and caruncles from dishes. *Rhytidoponera* species frequently prey or scavenge on other invertebrates, *Iridomyrmex* species have an omnivorous diet as do species of *Melophorus*, although ants in the latter genus differ from *Iridomyrmex* species in that they often include seeds as an important component of their diets.

The percentages of the three items in Petri dishes that were removed by ants during field trials are shown in Figure 1. The results clearly show that ants took virtually all of the unattached caruncles that were presented. The mean percentage of intact diaspores that were removed was greater than the mean percentage of (ecarunculate) seeds that were removed. The responses to ecarunculate seeds were also the most variable, since such seeds were often ignored but were sporadically removed from Petri dishes. Field observations of ants transporting *Adriana* seeds indicated that seeds are often rapidly located by ants once they fall to the ground and that ants usually transport seeds for distances of about a metre or less.

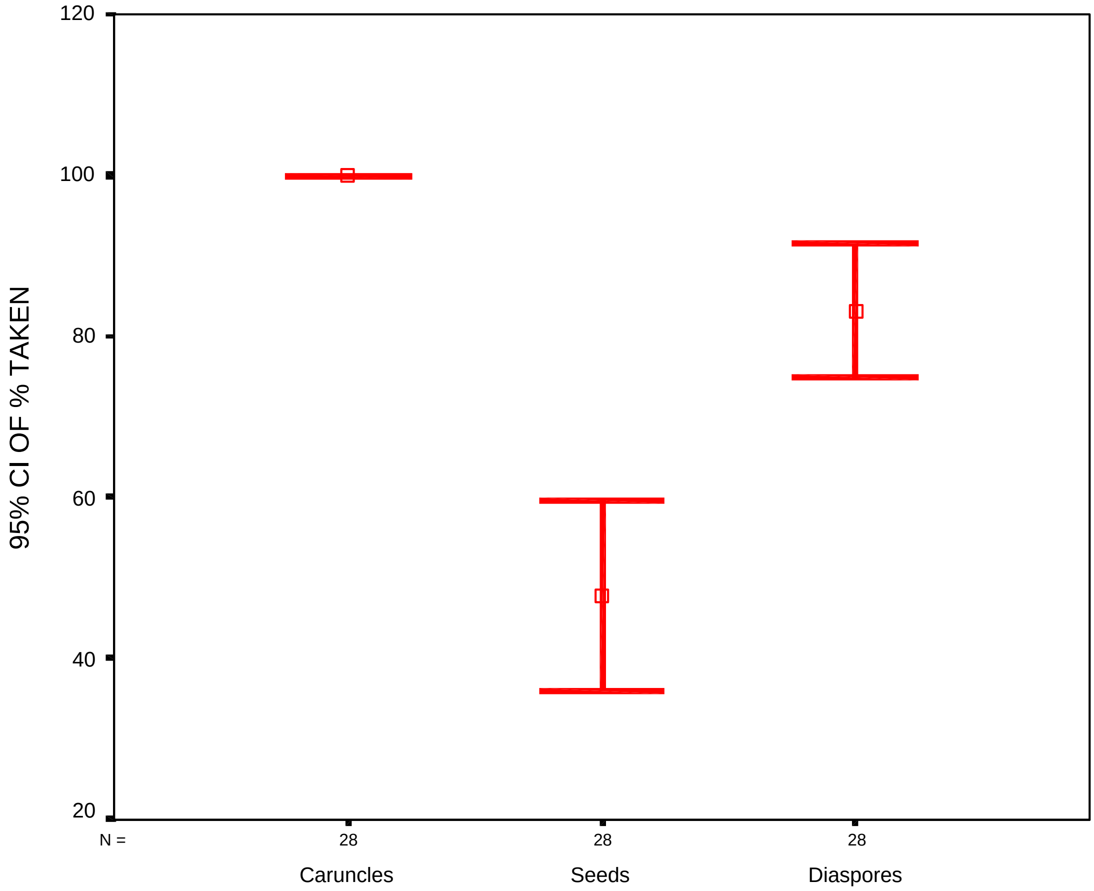


Figure 1. Removal of caruncles, seeds and diasporas from petridishes in field trials.

Seeds were excavated from ant nests at several localities to determine whether *Adriana* seeds were cached within the nests. Seeds of adrianas were found within nests of species of *Aphaenogaster*, *Iridomyrmex*, *Melophorus*, *Pheidole* and *Rhytidoponera*. Seeds in caches usually had both the caruncles and the outer layer of the seed coat removed, indicating the possible presence of a nutritious or attractive component in the seed coat, as well as in the caruncle. On average, the nests of *Rhytidoponera* species contained the greatest numbers of *Adriana* seeds.

Laboratory trials

To confirm and extend the results of the field trials, we established several laboratory colonies of *Rhytidoponera*, *Iridomyrmex* and *Melophorus*. The greater majority of lab trials were performed with colonies of *Rhytidoponera* since these ants behaved most naturally, and were the easiest to maintain, under laboratory conditions. Figure 2 presents boxplots of the percentages of caruncles, seeds (ecarunculate diaspores) and intact diaspores that were taken by laboratory colonies in eleven trials. The results parallel the field trials in that caruncles were taken more frequently than intact diaspores which in turn were taken more frequently than ecarunculate seeds.

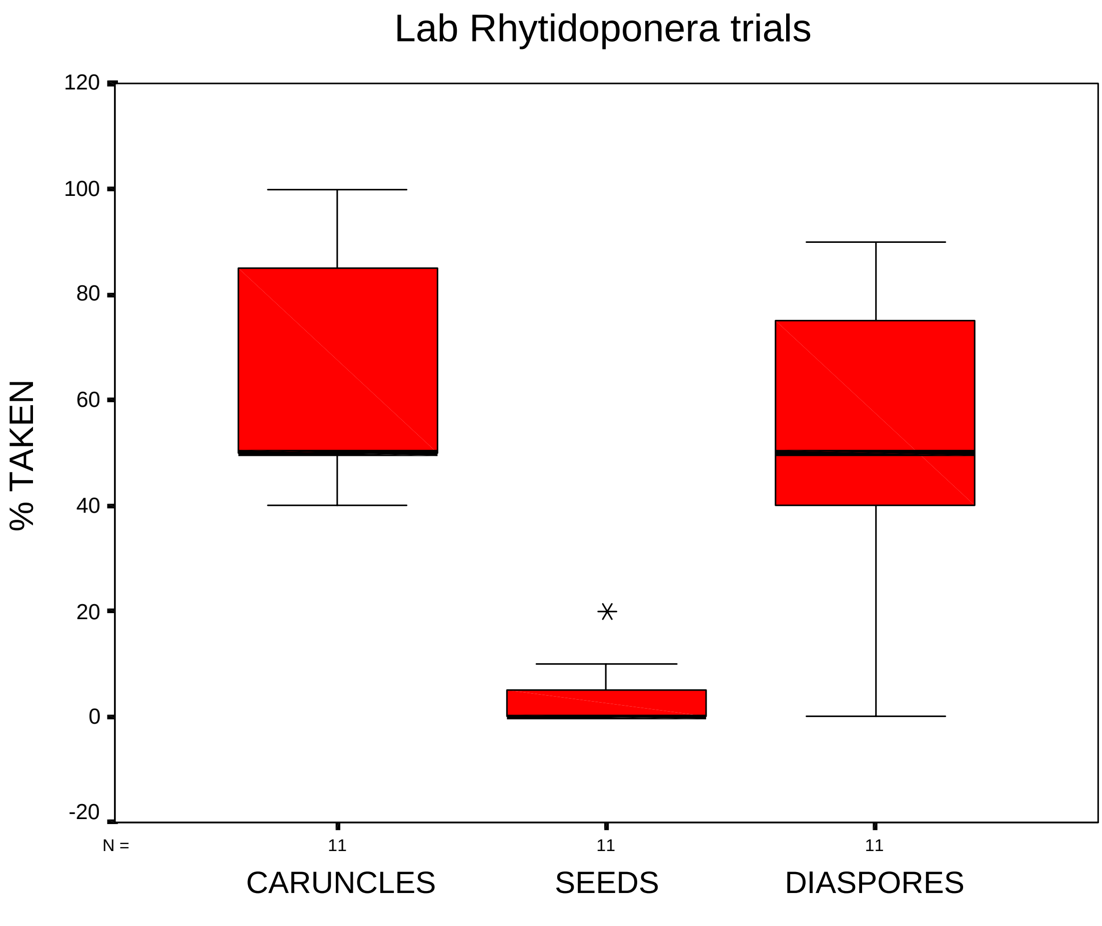


Figure 2. Removal of caruncles, seeds and diasporas by ants of the genus *Rhytidoponera* in laboratory trials

Together, the field and laboratory choice trials indicate that the caruncle is attractive to ants and that carunculate seeds were particularly attractive to the omnivorous *Rhytidoponera* and *Iridomyrmex* species.

Fatty acid constituents of euphorb caruncles

The fatty acid constituents of seeds of *Adriana* species and of *Ricinus communis* were analysed for us using gas chromatography by Mr. Mark Newman in the laboratory of Dr. Bob Gibson at the Flinders Medical Centre.

Seeds from plants of *Adriana quadripartita* were obtained from the following areas:- Marion Bay, Torrens Island and the Robe area (between Salt Creek and Robe) in South Australia (Figure 3). Seeds from plants of *Adriana tomentosa* var. *hookeri* were obtained from populations in Wyperfeld National Park in north-western Victoria (Figure 3).

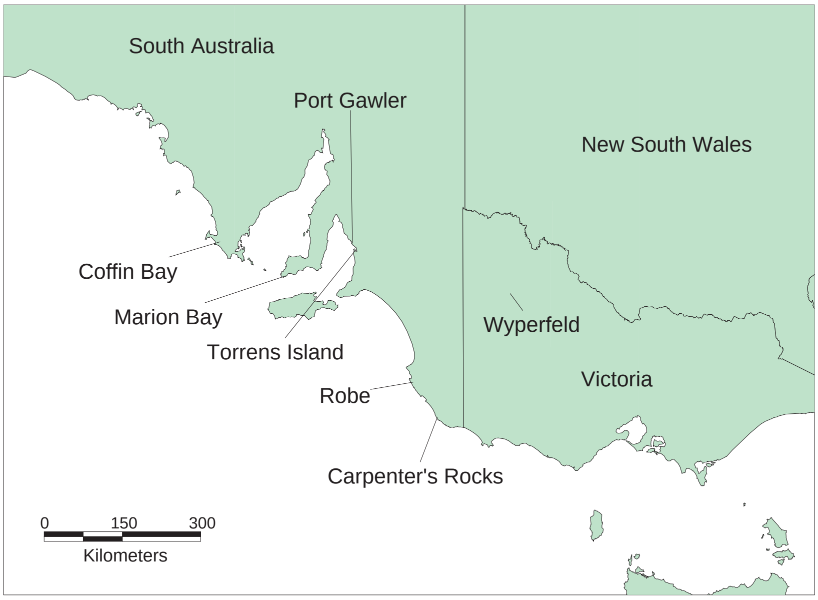


Figure 3. Areas used for collection of seed of *Adriana* species

Figures 4 and 5 present the relative proportions of the major fatty acids in the elaiosomes and seeds respectively, of *A. quadripartita*, *Adriana tomentosa* var. <u>*hookeri*</u> and *Ricinus communis*. The data depicted in the twelve bars on the right of these figures were obtained from a paper by Hughes et al. (1994) for comparison. Hughes et al. argue that the fatty acid composition of seed elaiosomes from a variety of plant species have convergently evolved so as to resemble the fatty acid composition of arthropods, thus making the elaiosomes (and seeds) especially attractive to carnivorous or omnivorous ants such as *Rhytidoponera*. Prior to our study, no comparative data were available from plants in the Euphorbiaceae. Figures 4 and 5 reveal that the fatty acid profiles from the two *Adriana* species are quite similar to each other and to those of the other elaiosome-bearing plant species examined by Hughes et al. (1994). Relative to the seeds themselves, caruncles contained higher proportions of 16:0 palmitic acid. Interestingly, the elaiosomes of *Ricinus communis*, the castor oil plant, are quite different in composition, containing substantial amounts of 18:3 linolenic acid. The seeds of *Ricinus* (average weight more than 200mg) are much larger than those of adrianas (average weight ~ 30mg) which themselves weigh more than the majority of ant-dispersed seeds tabulated by Hughes et al. (1991). This suggests that *Ricinus* seeds may not be myrmecochorous.

Figure 4

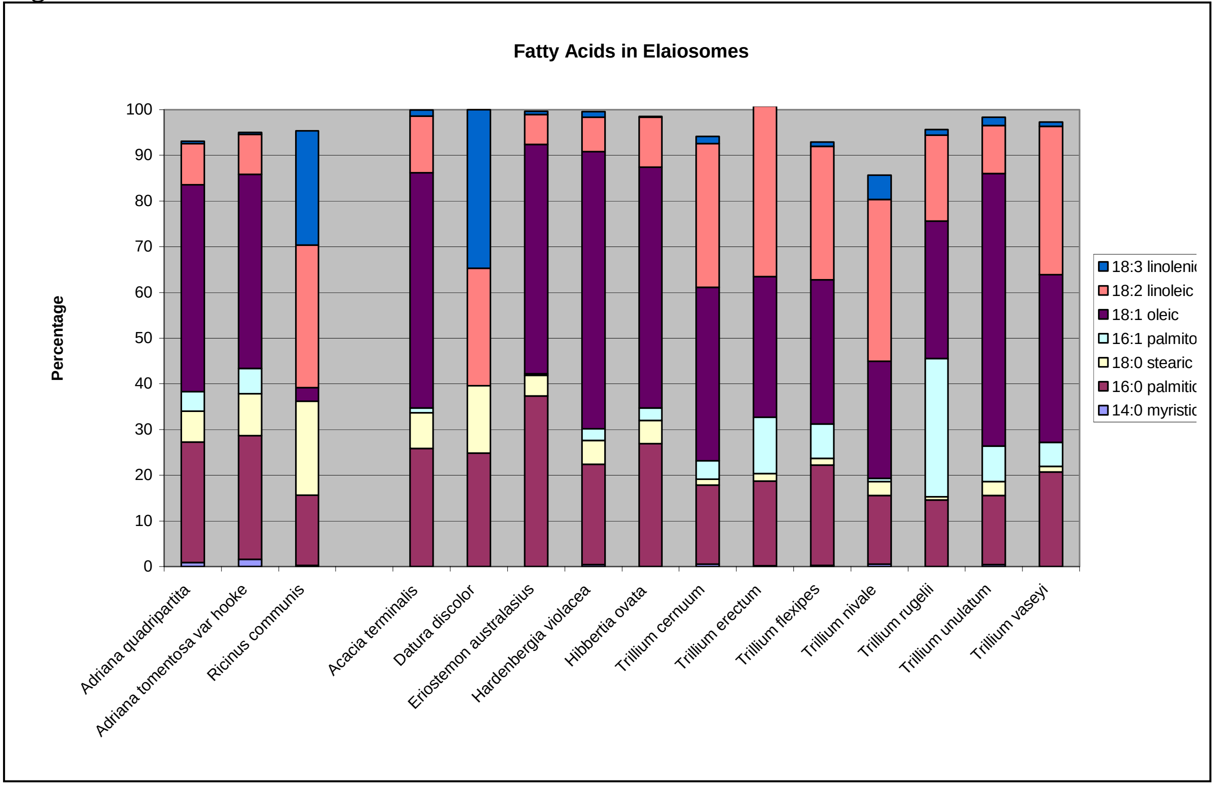
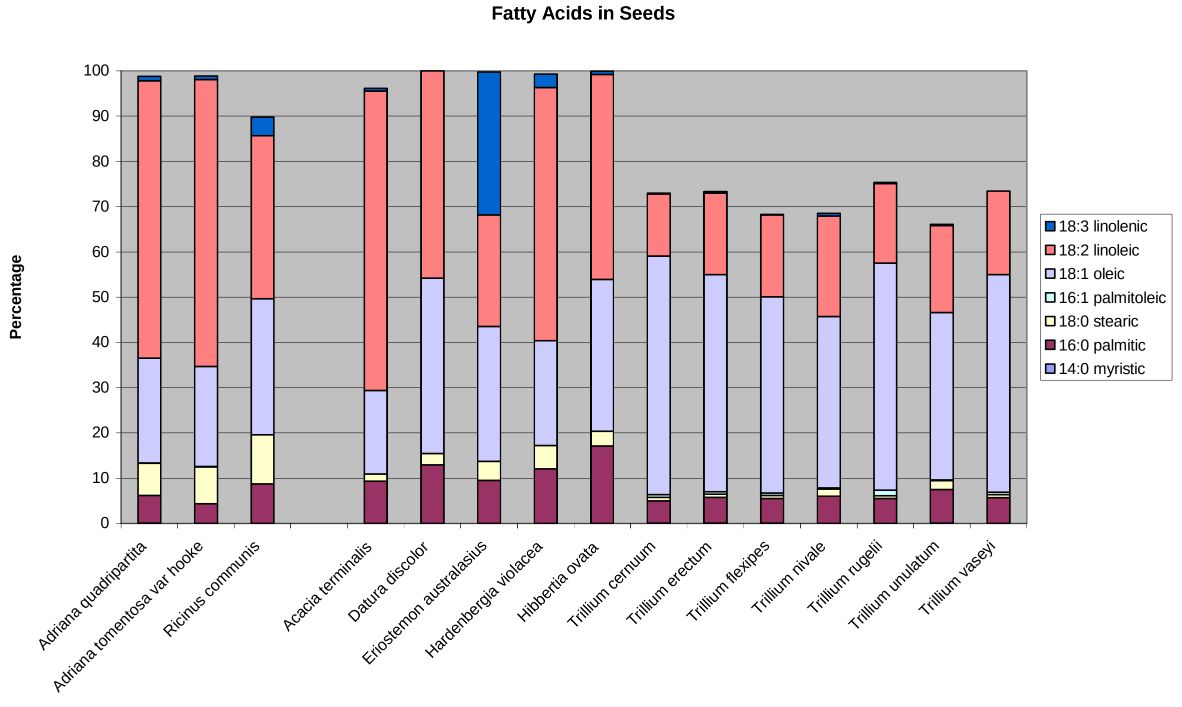


Figure 5



Figures 6 and 7 illustrate the levels of variation in the fatty acid composition of elaiosomes (caruncles) and seeds, respectively, among and within localities containing the two species of *Adriana*. The fatty acid composition is clearly quite uniform and there appears to be as much variation within populations or geographic areas as there is among populations or species. Since elaiosomes have been hypothesised to have evolved so as to resemble arthropods in their fatty acid composition (see above), we analysed the fatty acid composition of blowflies and found that they resembled *Adriana* caruncles in having relatively low concentrations (10%) of linoleic acid and intermediate concentrations (18%) of palmitic acid, in contrast to *Adriana* seeds which have relatively high concentrations (62%) of linoleic acid and relatively low concentrations (5%) of palmitic acid. A trial was conducted with a laboratory colony of *Rhytidoponera* ants in which the colony was offered equal numbers of *Adriana* diaspores, caruncles, ecarunculate seeds and blowflies. The colony tended to remove the caruncles first, then the flies and then the intact diaspores. Thus although the diaspores are clearly attractive to ants and have caruncles that appear to resemble arthropod prey items, ants can still differentiate between diaspores and insect prey of similar weights.

Figure 6

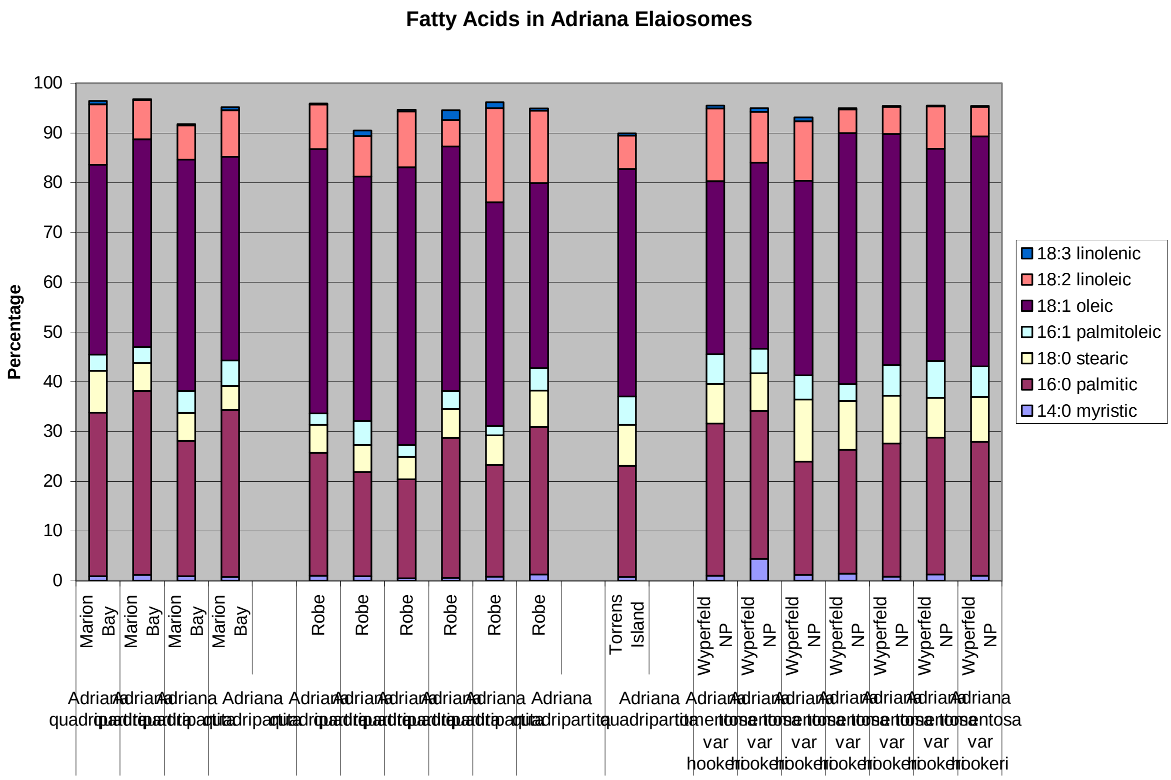
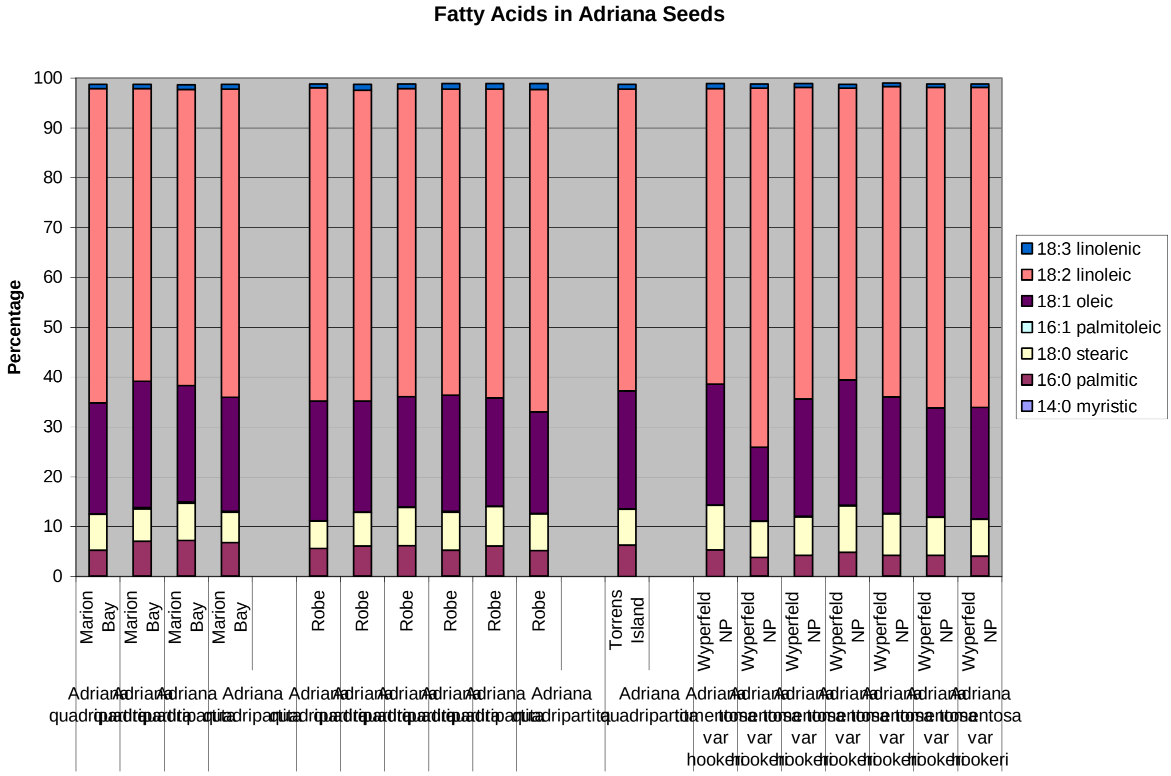


Figure 7



Variation in Seed and Caruncle Weights

The consequences of variation in the absolute and relative masses of the elaiosomes and seeds have been examined by several authors. Gunther and Lanza (1989) found that removal rates of seeds of three species of *Trillium* were not directly correlated with elaiosome mass, diaspore mass or with the elaiosome:diaspore mass ratio. Hughes and Westoby (1992) used elaiosomes glued to plastic beads to manipulate the above parameters and concluded that both diaspore mass and elaiosome:seed mass ratio contributed to seed removal rates. However they concluded that omnivorous ants (such as *Rhytidoponera*) responded primarily to the elaiosome:seed mass ratio, whereas seed-feeding ants (such as *Pheidole*) responded primarily to the diaspore mass. Mark and Oleson (1996) examined intraspecific variation in seed and elaiosome mass in a population of *Hepatica nobilis* (Ranunculaceae) and concluded that elaiosome mass, rather than the elaiosome:seed mass ratio was primarily influencing removal rates by ants. The present study appears to be the first to quantify the nature and extent of variation in elaiosome and diaspore masses within and among populations of a myrmecochorous plant.

Mature seeds were collected from at least three plants in each of several populations of *A. quadripartita* in coastal South Australia (Figure 3). Seeds and caruncles were weighed on a six-place microbalance. Figure 8 shows the relationship between seed (diaspore - caruncle) weight and caruncle weight across all populations. (Populations are coded with different colours). The relationship appears to be non-linear, with large seeds having proportionately larger caruncles. Analysis of covariance on log-transformed data indicates that the slope of the relationship between caruncle weight and seed weight does not differ significantly among the six sampled populations ($F_{5,164} = 1.95$, $P > 0.05$).

Figure 8

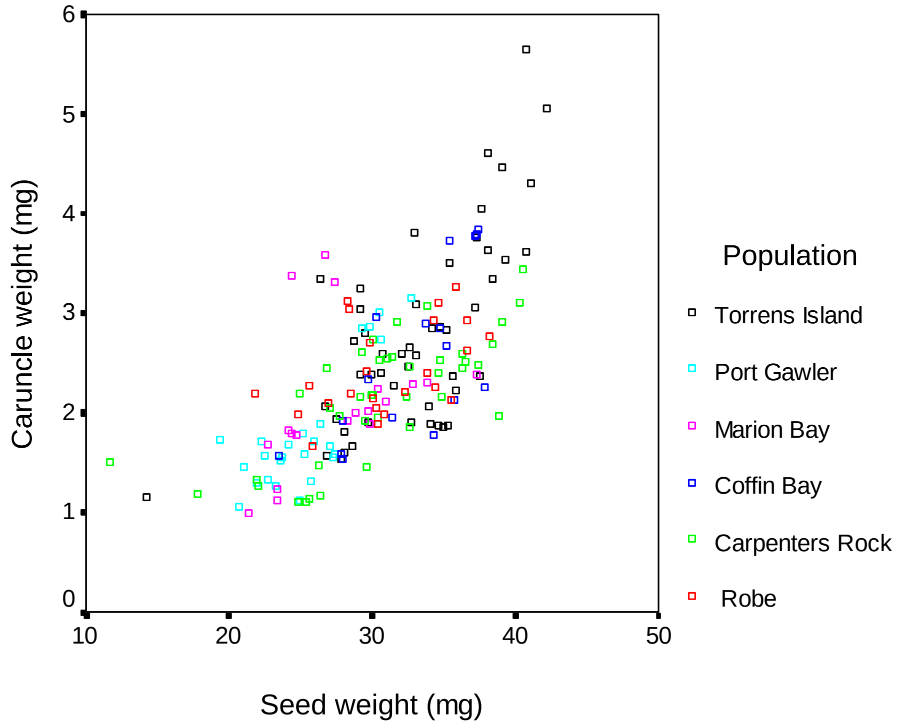


Figure 9

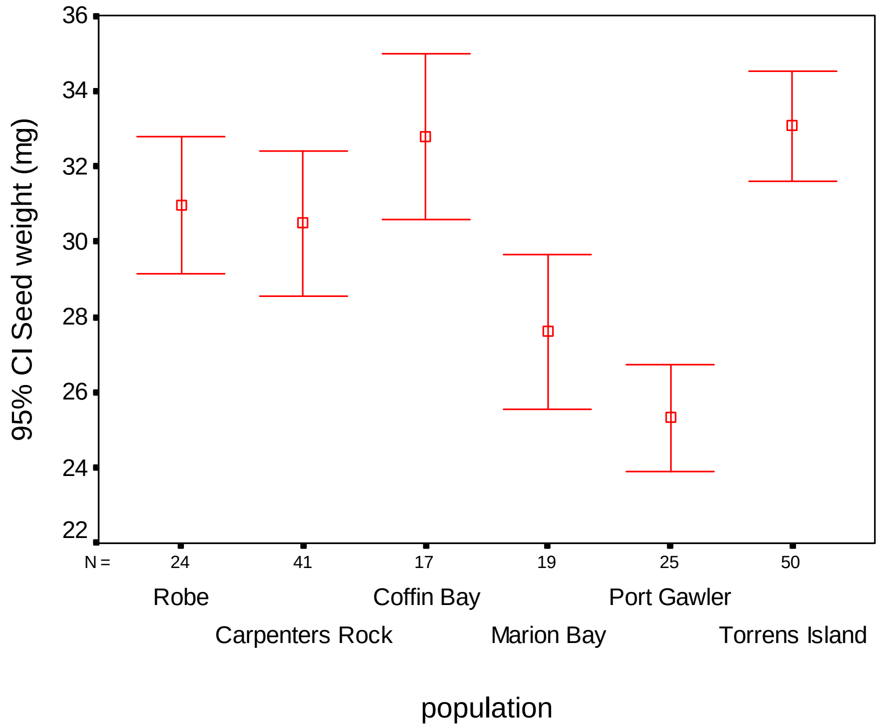


Figure 9 presents means and 95% confidence intervals of seed (diaspore minus caruncle) weight in the six sampled populations. Mean seed weight differed significantly among populations and among plants within populations (Analysis of Variance, $P < 0.05$, $P < 0.001$, respectively).

Figure 10 presents means and 95% confidence intervals of caruncle weight in the six sampled populations. Mean caruncle weight also differed significantly among plants within populations (Analysis of Variance, $P < 0.001$) but was not significantly different among populations (Analysis of Variance, $P = 0.232$), although the pattern of differences among populations was very similar to that for seed weights (Figures 9 and 10). Not surprisingly, the ratios of caruncle: seed mass ratios displayed a similar trend (Figure 11) and did not differ significantly among populations (Analysis of Variance, $P = 0.753$) although there was significant variation among plants within populations (Analysis of Variance, $P < 0.001$). We calculated variance components for the caruncle: seed mass ratio in order to quantify levels of variation in this trait and found that 77% of the variation arose from differences among plants within populations, 23% arose from differences among seeds from within individual plants and a negligible amount arose from differences among populations.

Figure 10

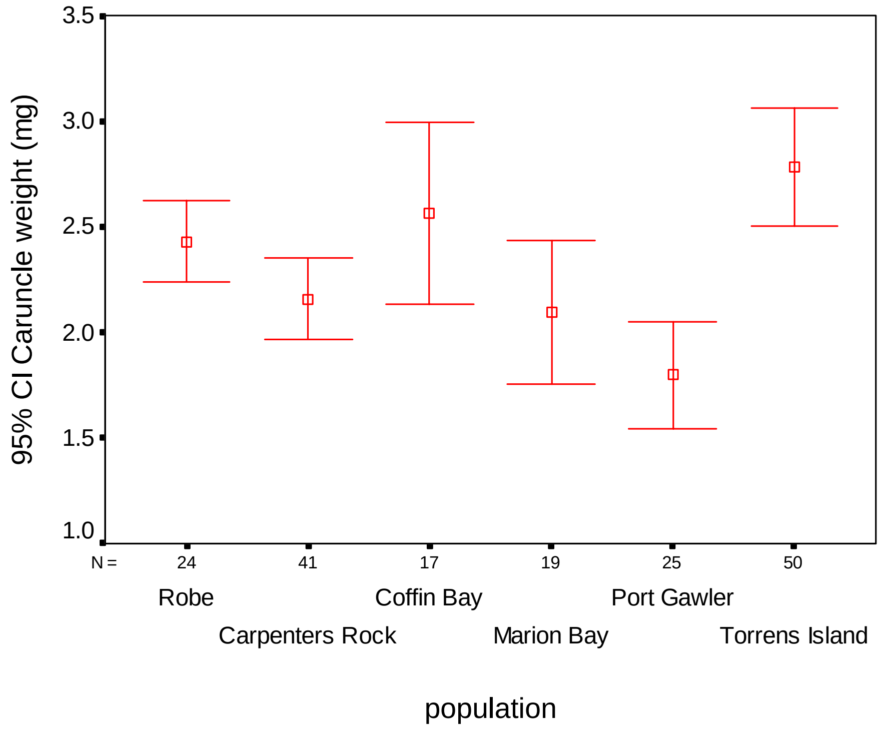
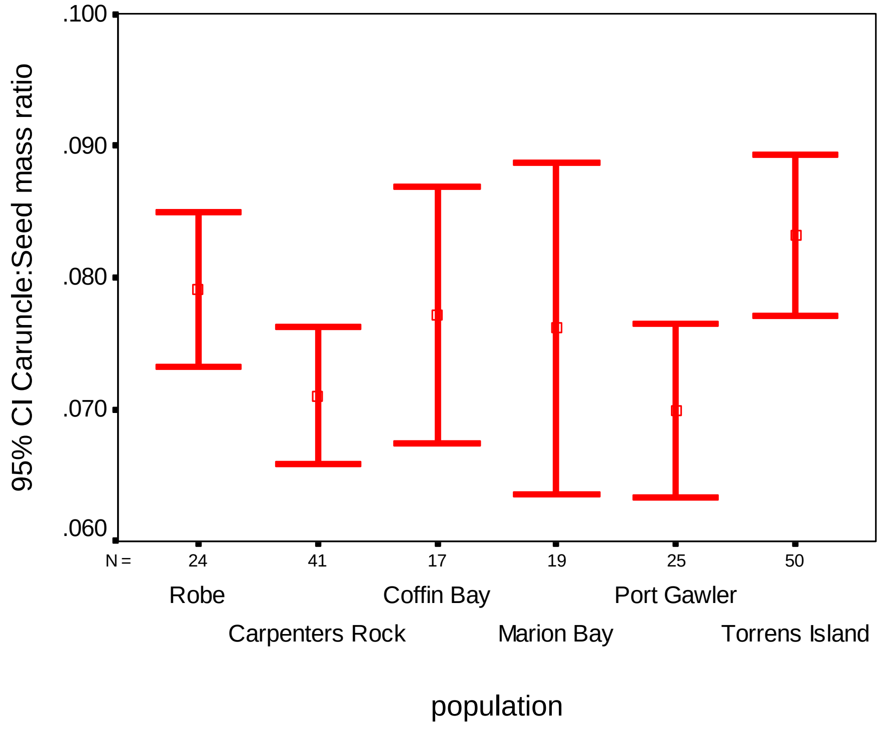


Figure 11



Seeds of *A. tomentosa var. hookeri* were collected from Wyperfeld National Park in Victoria. The means (and 95% confidence intervals) for the diaspore characteristics are similar to those measured from seeds of *A. quadripartita* and are shown below :-

Diaspore weight (mg)	Seed weight (mg)	Caruncle weight (mg)	Caruncle:seed mass ratio
29.15 (27.82,30.50)	26.90 (25.62,28.17)	2.26 (2.15,2.38)	0.086 (0.082,0.089)

Conclusions and Current Research

The carunculate seeds of adrianas are clearly attractive to ants of several different species and are frequently transported by ants to their nests. Analyses of the caruncle fatty acid constituents indicates that they resemble those of other myrmecochorous plant species and that there is little variation in the fatty acid composition of caruncles among *Adriana* species or among populations of adrianas.

Analysis of diaspore and caruncle weights has revealed that while there is significant variation in seed weight among sampled populations of *Adriana quadripartita*, the relationship between seed weight and caruncle weight does not vary among populations and hence the caruncle:seed mass ratio, which has been shown to be an important cue eliciting diaspore removal in other studies, does not vary significantly among the sampled populations. On the other hand, there is considerable variation among plants within populations in seed and caruncle weights, and in the ratio of these quantities. Such a pattern might arise if the factors influencing the evolution of the caruncle:seed mass ratio varied little over the sampled geographic range.

Current and future work is directed at determining the consequences of myrmecochory for seed germination and seedling establishment. This work involves germination trials in the lab and greenhouse and transplantation experiments in the field.

Outcomes

The work supported by the Flora Foundation grant has been the subject of papers presented at the joint meetings of the Australian Systematic Botany Society and Society of Australian Systematic Biologists at Adelaide, 1997 and at the meetings of the Society for the Study of Evolution at Boulder, Colorado, 1997. The work described above is also being prepared for publication in scientific journals.

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