that isolated hepatocytes from phenobarbital-treated rats are relatively resistant to the toxic effects of carbon tetrachloride (1.0 m*M*), bromobenzene (0.6 m*M*), and EMS (8 m*M*) for up to 4 hours when they are incubated at 37°C in Krebs-Henseleit buffer, *p*H 7.4, containing 2.6 m*M* Ca^{2+}. However, when similarly prepared hepatocytes are incubated at 37°C in Ca^{2+}-free Krebs-Henseleit buffer, *p*H 7.4, in the presence of either 1.0 m*M* carbon tetrachloride, 0.6 m*M* bromobenzene, or 8 m*M* EMS, approximately 90 percent of the cells are permeable to both NADH and trypan blue after 4 hours (Fig. 1, b and d), whereas control hepatocytes incubated in Ca^{2+}-free Krebs-Henseleit buffer alone are still approximately 70 percent viable after 4 hours (Fig. 1, b and d). It therefore appears that these three compounds are far more toxic to isolated hepatocytes in the absence of extracellular Ca^{2+} than in its presence. This result is inconsistent with the notion that an influx of extracellular Ca^{2+} is required as the final step in toxic injury of liver cells caused by carbon tetrachloride, bromobenzene, and EMS. Moreover, similar results are obtained when 0.1 m*M* EGTA, a highly specific Ca^{2+} chelator (*16*), is added to the Ca^{2+}-free Krebs-Henseleit buffer, *p*H 7.4. Thus, if carbon tetrachloride, bromobenzene, and EMS are toxic to isolated liver cells in Ca^{2+}-free buffer containing EGTA, it is apparent that the toxic liver cell injury caused by these compounds, as measured by two well-established parameters of liver cell viability, namely, trypan blue exclusion (*10*) and NADH penetration (*17*), is not dependent on extracellular calcium.

We therefore conclude that the influx of extracellular Ca^{2+} is not the final common pathway for the toxic death of isolated liver cells. We do not know why an influx of extracellular Ca^{2+} appears to be required for the toxic killing of cultured hepatocytes (*5, 7*) but not for freshly isolated hepatocytes. However, this discrepancy may be due to different effects of extracellular Ca^{2+} on attached and suspended liver cells. Moreover, our findings are still compatible with the hypothesis that the plasma membrane is a primary target in toxic liver cell injury, and that changes in its permeability to other ions, such as Na^+ and K^+, may be a crucial part of events that lead to cell death.

MARTYN T. SMITH
HJÖRDIS THOR
STEN ORRENIUS
Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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Competition for Dispersal in Ant-Dispersed Plants

Abstract. *Two closely related and coexisting plants (Chenopodiaceae) of the Australian arid zone are adapted for seed dispersal by ants. These facultatively perennial shrubs persist in saltbush communities largely as a result of highly directional dispersal to ant mounds, where conditions are favorable for establishment and growth. The two species grow predominantly on mounds and compete for dispersal to these favorable microhabitats.*

Active seed dispersal by animals is characteristic of many land plants and of most plants in certain habitats (*1*). Ecologists have speculated that these plants compete for dispersal services, but the evidence has been inferential. Fruits are often superabundant; when unharvested, they decline in quality and attractiveness, and many seeds are lost to seed predation and decay (*2, 3*). Differences among coexisting species in fruit type and fruiting phenology have been interpreted as evolutionary adaptations to minimize simultaneous demand for limited dispersal agents (*2, 4*). We provide the first quantitative data showing that competition for dispersal services affects plant population dynamics.

Myrmecochory is the dispersal of plant propagules by ants. In exchange for dispersal services, myrmecochorous plants provision their diaspores (dispersal units) with ant-attractive food bodies. Often, the advantage to the plant is a reduction in the rate of parasitism or the intensity of competition as seeds are removed from the vicinity of the parent (*5*). Myrmecochores are remarkably common in the flora of Australia; an estimated 1500 species occur in dry heathlands and sclerophyll forests alone (*6*). Our studies (*7, 8*) suggest that in the Australian arid zone, myrmecochory functions primarily to position seeds in favorable microhabitats for establishment and growth. We report on investigations of arid zone myrmecochores in two closely related genera, *Sclerolaena* and *Dissocarpus* [united until recently (*9*) in *Bassia* (Chenopodiaceae)]. We demonstrate that (i) myrmecochores are differentially abundant on ant mounds, where diaspores are concentrated by the foraging activities of ants; (ii) ant mounds represent favorable microhabitats for myrmecochorous and nonmyrmecochorous plants alike; (iii) myrmecochores are relatively poor competitors when growing away from mounds; and (iv) mound populations of one myrmecochore are reduced significantly in the presence of a second species of ant-dispersed plant.

Sclerolaena diacantha and *Dissocarpus biflorus* var. *biflorus* are common ant-dispersed shrubs in the saltbush communities of arid central Australia (*7*). The diaspores of both species consist of

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Table 1. Chi-square matrix of comparisons of population density classes of myrmecochores on mounds from five sites. Chi-square values are given with significance levels below; the degrees of freedom are 2 in each comparison. Values in parentheses are the numerical difference in χ^2 value and significance level for this difference for the comparison of the same sites above and below the diagonal. N.S., not significant.

Locality	*S. diacantha* common			*S. diacantha and D. b. biflorus* common	
	Saloon (west)	Conservation	Saloon (central)	Warrens	Saloon (east)
Saloon (west)		0.61 N.S.	0.28 N.S.	9.91 $P < .01$	7.47 $P < .05$
Conservation			1.07 N.S.	13.37 $P < .01$	10.21 $P < .01$
Saloon (central)				7.20 $P < .05$	5.10 $P < .10$
Warrens	2.19 (7.72) N.S., $P < .05$	0.27 (13.10) N.S., $P < .01$	2.70 (4.80) N.S., $P < .10$		0.40 N.S.
Saloon (east)	0.00 (7.47) N.S., $P < .05$	0.90 (9.31) N.S., $P < .01$	0.30 (4.50) N.S., $P < .10$		

seeds encased in fruits and hardened woody perianths. Unlike the diaspores of nonmyrmecochorous congeners, those of *S. diacantha* and *D. b. biflorus* have basal cavities containing a soft, moist food mass to which a variety of ants are attracted. At Fowlers Gap in northwestern New South Wales, Australia, a large, abundant and primarily predatory ant, *Rhytidoponera* sp. B, is the principal dispersal agent for both plant species (*7*). Workers forage avidly for diaspores and invariably carry them to the nest to extract the relatively inaccessible food material. Ants later dispose of many undamaged diaspores in refuse heaps on their mounds. In contrast to closely related but nonmyrmecochorous plants growing at the same localities, myrmecochores are differentially abundant on *Rhytidoponera* mounds. At each of five study sites, we compared plant densities in 0.25-m^2 quadrat samples on and off *Rhytidoponera* mounds. A square sampling frame was alternately centered over a nest and tossed over the shoulder to a random location (20 paired samples per locality). In all possible comparisons, densities of myrmecochorous species were significantly greater on mound quadrats (*10*).

Associations of the two myrmecochores with mounds reflect both the differential success of plants in these microhabitats and highly directional dispersal to mounds. Both *S. diacantha* and *D. b. biflorus* are facultatively perennial; they survive longer and grow larger on *Rhytidoponera* mounds (Fig. 1), probably because potentially limiting nutrients are more concentrated in mound soils than in the surrounding alluvial crust (*11*). Our data suggest that *S. diacantha* persists in saltbush shrublands primarily by virtue of highly successful dispersal to these favorable microhabitats. In contrast to *S. ventricosa*, a closely related species that is not ant-dispersed, *S. diacantha* is a poor competitor when growing away from mounds. Off mounds, *S. diacantha* comprises a significantly smaller proportion of plant biomass ($r = -.40$, $P < .05$) and *S. ventricosa* a significantly larger proportion ($r = .52$, $P < .01$), as total plant biomass increases (*12*). Although mean densities of the two species are similar (1.53 *S. diacantha* versus 1.63 *S. ventricosa* per 0.25 m^2), individuals of *S. diacantha* are typically stunted and in poor condition. For quadrats sampled on *Rhytidoponera* mounds, the proportionate biomass of the myrmecochore increases slightly but insignificantly with total plant biomass, and no pattern is apparent for *S. ventricosa*.

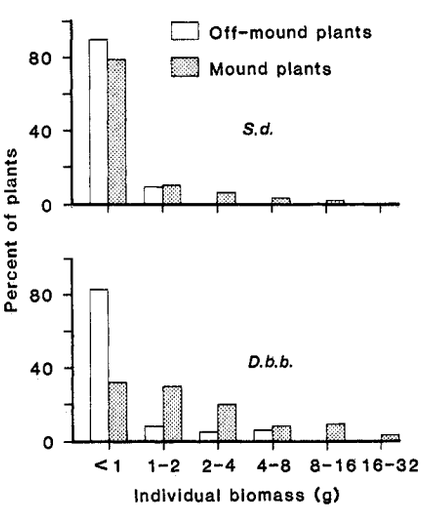

Fig. 1. Size frequency distributions (dry weight) of myrmecochores, *S. diacantha* (*S.d.*) and *D. b. biflorus* (*D.b.b.*), growing on and off *Rhytidoponera* mounds at two sites: Saloon (central) paddock (*S.d.*) or Salt 1 paddock (*D.b.b.*). In χ^2 tests comparing sizes of plants in mound and off-mound quadrats: $P << .001$ for *S.d.* [N (mound) = 155, N (off-mound) = 46]; $P << .001$ for *D.b.b.* [N (mound) = 222, N (off-mound) = 59]. For a nonmyrmecochore, *S. ventricosa*, $P < .002$ ($N_m = 52$, $N_o = 49$) in a similar comparison at Saloon (central) site.

Despite substantial variation in myrmecochore densities on mounds (*13*), plants and propagules are sufficiently crowded on some mounds to suggest that competition is intense. We investigated the possibility that mound population densities of *S. diacantha*, the myrmecochore common to all five sites, were reduced where *D. b. biflorus* was present. Recognizing the mound-to-mound variability in plant cover, we first classified 20 mounds at each of the five sites into three categories according to the density of *S. diacantha*: low density mounds had 0 to 9 plants, moderate density mounds had 10 to 19 plants, and high density mounds had 20 or more plants per 0.25-m^2 quadrat. At the two localities where *D. b. biflorus* occurred, we also classified mounds into the same three categories on the basis of the combined densities of both myrmecochore species.

Table 1 is a χ^2 matrix of pairwise comparisons of the density distributions of myrmecochores at the five study localities (*14*). Above the diagonal, χ^2 values compare population density classes of *S. diacantha* on mounds, both at sites where it grows alone and where *D. b. biflorus* is also present. In three comparisons possible among sites where *S. diacantha* is the only common myrmecochore (individuals of *D. b. biflorus* ≤ 3 in total census), there are no statistically significant differences between density class distributions; likewise, there is no statistical difference in density class distributions between Warrens and Saloon (east), two sites where both myrmecochores are common. However, the density distributions of *S. diacantha* populations differ significantly in five of six possible comparisons between localities containing only one myrmecochore and sites where both myrmecochores occur. The sixth comparison [Saloon (central) and Saloon (east)] borders on statistical significance. There are more mounds with low densities of *S. diacantha*, and fewer mounds with moderate and high densities at localities where *D. b. biflorus* is common (*15*).

Below the diagonal in Table 1, comparisons between sites with one myrmecochore and localities with both myrmecochores are repeated for population density classes of *S. diacantha* and *D. b.*

biflorus combined. At all localities, the density distributions of total myrmecochore populations on mounds are statistically indistinguishable. These results suggest that the two myrmecochorous chenopods are competing for a spatially limiting microhabitat (*16*).

What is the mechanism of the competition? Depression of *S. diacantha* populations in the presence of *D. b. biflorus* is probably mediated both by competition for dispersal to ant nests and by competition for space on mounds. We cannot yet assess the relative contributions of these processes or evaluate the factors favoring one competitor over another (*17*). It seems clear, however, that competition for dispersal agents may be important in regulating the composition of plant communities in this and similar floras where plant establishment depends on highly directional transport of seeds to favorable microhabitats.

D. W. DAVIDSON
Department of Biology,
University of Utah,
Salt Lake City 84112

S. R. MORTON*
School of Biological Sciences,
University of Sydney 2006,
New South Wales, Australia

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10. Student's *t*-test: $P < .02$ to $P < .001$ ($N = 5$ sites) for *S. diacantha*; $P < .005$ and $P < .001$ ($N = 2$) for *D. b. biflorus*. A second variety of *D. biflorus* (*D. b.* var. *cephalocarpus*) was not ant-dispersed and did not grow in association with ant mounds ($N = 2$). Likewise, *S. ventricosa*, a nonmyrmecochore related to *S. diacantha*, was not associated with mounds ($N = 5$).
11. In general, soils of the Australian arid zone are old, leached, and nutrient-poor. Depending on depth, concentrations of available nitrogen average from more than 100- to more than 200-fold higher on mounds than off mounds, and available phosphorus is more than two to three times more concentrated [$P << .0005$ for each comparison (*7*)]. Soils are also less compacted on mounds ($P << .0005$) and may allow greater water penetration and storage.
12. Off the mounds, bare scalds alternate with patches of vegetation, but our sampling scheme reduced microhabitat heterogeneity among samples. We sampled contiguous quadrats (0.25 m^2) along a transect and harvested plants only from those quadrats containing individuals of both species. Plants from both mound and off-mound quadrats were oven-dried to constant weight. For each quadrat sample, we measured the above-ground biomass of *S. diacantha* and *S. ventricosa* and the combined above-ground biomass of all other plants.
13. Mounds differ in both size and nutrient concentration (*7*) as well as in proximity to a source of diaspores.
14. Between sites, mean plant densities per mound quadrat ranged from 7.50 to 23.80, with the densities of nonmyrmecochores averaging ≤ one individual per mound at each site. We adjusted plant densities at each locality to the mean density across all sites (17.15 plants/mound). By standardizing the data in this way, we assume implicitly that the effect of an individual of *S. diacantha* on an individual of *D. b. biflorus* (and vice versa) is the same at all five sites, regardless of plant density. Variability in plant density probably parallels between-site differences in the suitability of growth conditions, such as access to limiting water and nutrients as a function of rainfall or soil type.
15. Over the three sites where *D. b. biflorus* was absent, there were averages of 9.00 low density, 5.33 moderate density, and 5.67 high density populations on mounds, in comparison to means of 15.50, 2.00, and 2.50, respectively, for sites at which *D. b. biflorus* was present.
16. On mounds, individuals of *D. b. biflorus* have an average of 1.18 times greater biomass than individuals of *S. diacantha*. Adjustment of mound populations by this equivalency factor does not alter the distribution of density classes at any site.
17. Differences in the diaspores of *S. diacantha* and *D. b. biflorus* may influence their relative success in becoming established. Those of *D. b. biflorus* contain more food material (both per propagule and per gram of diaspore) than diaspores of *S. diacantha*, and *D. b. biflorus* has two seeds per propagule in contrast to one in *S. diacantha*.
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* Present address: Alligator Rivers Region Research Institute, Office of the Supervising Scientist, Jabiru, New Tasmania 5796.

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Reversal of Diabetes by Islet Transplantation: Vulnerability of the Established Allograft

Abstract. *Nonspecific stimulation of the immune system of CBA mice carrying a functional BALB/c islet allograft failed to trigger graft rejection. Only three of six animals rejected their graft when injected intravenously with 10^5, 10^6, and 10^7 peritoneal cells of BALB/c origin over a 3-month period commencing 100 days after transplantation.*

Organ culture of pancreatic islets before transplantation can facilitate allograft acceptance by normal recipient animals (*1, 2*) or by recipients conditioned with a single dose of antiserum to lymphocytes at the time of transplantation (*3*). Such allografts are functional and reverse streptozotocin- or alloxan-induced diabetes (*1–5*). These findings indicate that pancreatic islet transplantation might be used to reverse insulin-dependent diabetes in humans. However, before considering clinical application of this technology, we need to know more about the susceptibility of the established allograft to rejection.

Organ culture before transplantation reduces the immunogenicity of islet, thyroid, and parathyroid tissue (*5*) but does not destroy tissue antigens; cultured allografts are consistently rejected when the recipient is immunized with lymphoreticular cells of donor origin at the time of transplantation (*5, 6*). This reduction in tissue immunogenicity is thought to result from a loss, or inactivation, of lymphoreticular stimulator cells during culture in an oxygen-rich atmosphere (*7–9*). T cell activation by alloantigens is very efficient when the stimulator cell provides both alloantigens and a source of costimulator activity, the second signal required for T cell activation (*10*). When these stimulator cells are removed from the tissue before transplantation the tissue retains recognizable antigens, but these are much less immunogenic in the recipient (*5, 7*).

While cultured allografts can be transplanted without a need for suppression of the recipient's immune system, such grafts are constantly under the threat of rejection. Nonspecific stimulation of the recipient's immune system could raise the level of costimulator activity and trigger irreversible rejection. In the case of transplants in humans, transfusion of blood for some therapeutic reason unrelated to the tissue transplant could trigger rejection if the transfused blood carried histocompatibility antigens similar to those in the graft. In this study we investigated the extent of these threats to the continued survival of an established allograft of cultured islet tissue in the mouse.

We first investigated the effect of nonspecific stimulation of the immune system on islet allograft function. CBA mice were made diabetic by intravenous injection of streptozotocin (300 mg/kg). Approximately 2 weeks later animals with blood sugar concentrations greater than 20 mmole/liter were given cultured

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