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REGULATION OF AN INSECT POPULATION UNDER BIOLOGICAL CONTROL[1]

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Abstract. California red scale is suppressed to very low densities by the parasitoid *Aphytis melinus*. The system also appears stable. We report on an experimental test of the hypothesis that stability is caused by a refuge for scale. In a grapefruit grove in southern California in 1984-1985, the bark in the interior part of the tree provided a partial refuge from parasitism. Scale were ≈100 times denser there than in the exterior of trees. In a field experiment, we removed Argentine ants from some blocks of trees to test whether (1) ants caused the refuge by interfering with *Aphytis* and (2) the expected reduction in scale density in the refuge would lead to an unstable interaction in the exterior. We also tested for density-dependent parasitism, host mutilation, and predation by analyzing data from samples and from scale placed in the field.

The temporal variability of the scale was at the low end of the range recorded in field populations. The experiment provided some evidence in support of the refuge hypothesis. The population in the refuge fluctuated much less than that in the exterior. Ant exclusion led to increased parasitism and lower scale density in the interior, and to increased fluctuations in abundance in the refuge and exterior. However, these changes were relatively small and perhaps temporary, suggesting that (1) ants are not the main cause of the refuge and that (2) we did not reduce the refuge density enough to determine whether the system would go unstable in the absence of the refuge population.

Parasitism, host mutilation, and predation rates on scale showed no temporal density dependence, either direct or delayed, though detection of such patterns is difficult. Possible alternative stabilizing mechanisms include size-dependent interactions between red scale and *Aphytis*.

Key words: Aphytis; host feeding; parasitoids; population regulation; red scale; refuge; southern California; stability.

INTRODUCTION

California red scale (*Aonidiella aurantii*), an insect pest of citrus, is under successful control by the parasitoid *Aphytis melinus* in many parts of the world (DeBach et al. 1971, Rosen and DeBach 1979). In inland valleys in southern California (within ≈40 km of the coast), the interaction appears to be well regulated in the sense that the populations persist over long periods, fluctuations in density are bounded within a narrow range, and mean population densities show no

trends over long periods (e.g., DeBach 1958, DeBach et al. 1971, Reeve and Murdoch 1985).

Reeve and Murdoch (1986), Murdoch et al. (1989), and Yu et al. (1990) showed that the bark in the interior of lemon and grapefruit trees provided a partial refuge where parasitism by *Aphytis* was about an order of magnitude lower than in the exterior part of the tree (parasitism by *Encarsia perniciosi*, a species considered to play a minor role in control, was also lower in the interior). Presumably as a consequence, scale were almost 100 times more dense in the refuge. Casual observations in other groves suggested that the refuge was absent when Argentine ants were absent or rare.

Table 1. Development times, in degree-days, for red scale and *Aphytis*. Data are averages from experiments run at 20°, 25°, 26.7°, and 30°C by Yu (1986).

Development time (degree-days)					
Red scale					
Female		Male		*Aphytis*	
Instar 1	106.5	Instar 1	106.5	Egg	42.7
Molt 1	70.5	Molt 1	70.5	Larva	88.1
Instar 2	75.8	Instar 2	50.5	Prepupa	19.3
Molt 2	92.5	Male 2	77.3	Pupa	93.4
Instar 3	103.8	Prepupa	20.9		
Mature female	202.8	Pupa	49.3		
Total	651.9		375.0		243.5

The ants have runs along the trunk and main branches in the interior of trees and, though they do not tend red scale, they might create the refuge in the interior by interfering with searching adult *Aphytis*. These observations were also consistent with DeBach's (1958) experimental evidence that red scale were more abundant in the presence of Argentine ants. An alternative or additional cause of low parasitism in the refuge could be that the bark color in the interior is not attractive to *Aphytis* (Gregory 1985). Scale are also smaller on average in the interior, and *Aphytis* is known to prefer larger scale. With scale size held constant, however, the rate of parasitism was lower in the interior (Luck and Podoler 1985, Yu 1986, Walde et al. 1989).

Reeve and Murdoch (1986) suggested that the refuge population in a lemon grove might stabilize the otherwise unstable interaction in the exterior region of the tree by leaking crawlers to the exterior at a relatively steady rate. We refer to this hereafter as the refuge hypothesis. They supported this idea by showing that temporal variability in the exterior population was lowest in instar 1 and increased in the successive scale stages. Variation in parasitism by *Aphytis*, unrelated to scale density, could help to account for this increasing temporal variability.

This paper reports on a field experiment to investigate the following questions. Is the refuge population less temporally variable than that in the (nonrefuge) exterior sections of the tree and do ants create the refuge? We experimentally removed ants and predicted that parasitism by *Aphytis* in the refuge would increase and scale density would decrease. Is the refuge scale population a source of stability for the interaction in the exterior? The predicted reduction in scale density in the refuge should lead to reduced immigration of scale in the exterior population, which should fluctuate more as a consequence. Do dynamics differ between the refuge and exterior and, finally, do other potential stabilizing mechanisms exist, including temporal density dependence of parasitism and other causes of mortality?

Natural History and Methods

Life histories

Details of the scale's natural history are in Ebeling (1959) and are summarized in Murdoch et al. (1989)

and Hare et al. (1990). Female scale can produce as many as 100–150 crawlers in their lifetime. Female scale pass through three instars and two intervening nongrowing "molt" stages. Male and female scale differentiate morphologically midway through instar 2. Males pupate at the end of instar 2 and emerge as winged adults coincident with the presence of virgin female scale (i.e., instar 3); males live for only ≈24 h (Moreno and Kennett 1985). There are two overlapping scale generations per year in the study area.

Instar 3 (virgin females) become receptive to males after developing for ≈100 degree-days (Table 1). Insemination is followed by morphological changes that distinguish the mature female. Once inseminated, mature females require ≈200 degree-days to produce the first crawler, so this is normally the longest prereproductive stage. Total development time in the laboratory, from settling of a female crawler to the time it produces its first crawler, is ≈650 degree-days (Table 1). Total development time in the field may be longer (J. D. Hare, *personal communication*).

Aphytis melinus biology is described by Rosen and DeBach (1979). *Aphytis* parasitizes mainly large female instar 2, female instar 3, and male instar 2 (Abdelrahmen 1974, Rosen and DeBach 1979). Larger scale may receive more than one egg (Luck et al. 1982). Male offspring result from unfertilized and female from fertilized eggs (Luck and Podoler 1985, Opp and Luck 1986). Eggs are laid under the scale cover on the scale body. Most female *Aphytis* come from larger instar 3 hosts (Luck and Podoler 1985, Yu 1986). *Aphytis* also frequently kills hosts, without parasitizing them, by probing the scale body with its ovipositor. Following probing the parasitoid may, or may not, feed on the host haemolymph, but we designate all such attacks as "host mutilation" since hosts that have been fed upon cannot be distinguished in samples from those that were simply probed. Host mutilation occurs on all stages except second molt, mature and crawler-producing females, and male pupal stages (Abdelrahmen 1974, Yu 1986). *Aphytis* has about three generations for each scale generation (Table 1).

Scale in our study area are also attacked by the parasitoid *Encarsia perniciosi* (Rosen and DeBach 1978),

which is thought to be a minor contributor to control. *Encarsia* parasitizes all stages of scale, but mainly instars 1 and 2 (Yu et al. 1990). *Encarsia* parasitism of instars 1 and 2 cannot be recognized in field samples, however, until the parasitoid larva causes the scale's epidermis to harden and transform into a semitransparent case beneath the scale cover (i.e., a "mummy"), visible when the parasitized scale is a molt 2, instar 3, or mature female. It was thus not possible to estimate the true parasitism rate by *Encarsia* in our samples because we cannot estimate the number of instars 1 and 2 that are parasitized. An index of parasitism is: (total number parasitized)/(total number parasitized + number unparasitized molt 2, instar 3, and mature females). *Encarsia* has ≈ 2 generations per scale generation (Yu and Luck 1988, Yu et al. 1990).

Experimental design

The study was done in a grapefruit grove in a large citrus-growing region in the Santa Paula Valley, Ventura County, 16 k west-southwest of Fillmore, California (Murdoch et al. 1989). Trees were 2.7–3.5 m in diameter and 3.3–4.0 m high. The experiment had three treatments: control (no treatment), "skirt control" (the tips of bottom branches were trimmed to remove their contact with the ground and reduce the movement of ants onto trees), and "skirted and ant exclusion." In this last treatment, the bottom branches were trimmed and, to exclude ants, the bottom 15 cm of the trunk were sprayed until run-off with a 2% (actual ingredients) liquid chlorpyrifos 4 emulsifiable concentration to which was added a 0.25% spreader-sticker (Western Farm Service, Walnut Creek, California) (Moreno et al. 1987).

Each replicate of a treatment consisted of a plot containing seven rows of seven trees each; plots were separated from adjacent plots by at least two rows of trees. All trees within a plot were subjected to the same treatment. Two trees were chosen to be sampled from the eight trees immediately surrounding the central tree in each plot. The first tree was chosen at random and the second tree was directly opposite the first. The plots were arranged in 4 blocks, each containing one plot from each treatment, and treatments were assigned to plots at random within a block. The first sampling date was 19 June 1984, and the treatments were applied on 14 August 1984. Ants did not return to treated areas, but as a precaution the spray treatment was repeated on 8 April 1985.

We monitored the effectiveness of ant exclusion every 2 wk in summer and every month the rest of the year by counting for 2 min on each date the number of ants moving up the base of each tree trunk. In the period before spraying, the numbers of ants did not differ between trees designated to be sprayed and the controls ($t_{22} = -1.22$, $P = 0.24$). The number of ants counted on sprayed trees averaged over the period following spraying (mean $\pm$ 1 SE = 1.73 $\pm$ 0.37) was reduced to a small fraction of that on the control trees (72.15 $\pm$ 7.91).

We would not expect treatment effects on scale to occur instantaneously. We therefore defined posttreatment dates for parasitism rate as starting 250 degree-days (roughly the development time of immature *Aphytis*) after the actual treatment. This removed from consideration any parasitism events that occurred before the treatment. Similarly, we defined posttreatment dates for scale density as beginning 600 degree-days after the actual treatment (roughly the time required for maturation and reproduction of scale that might have experienced reduced parasitism).

The experiment was brought to an early end when the grove owner sprayed the trees with insecticide at the end of 1985.

Sampling methods

Individual grapefruit twigs grow one or two flushes per year. Red scale are distributed over the entire tree, from the newest flush of growth to the oldest part of the trunk. Scale density increases and parasitism decreases along this gradient (Murdoch et al. 1989). We designate the newest four flushes of growth as "exterior": they bear almost all the leaves and the fruit, and the bark contains chlorophyll. We designate as "refuge" all substrate interior to the fourth flush. Here the bark typically lacks chlorophyll and the branches are woody.

The exterior population was sampled by taking twigs from each tree, at randomly chosen heights and compass directions. We took 4 (June 1984–March 1985 and November–December 1985) or 2 (April–October 1985) twigs, each consisting of the four newest flushes. Trees were sampled every 2 wk from May through October and monthly from November through April.

In a previous paper reporting the mean abundance on different substrates over the entire experiment, we expressed scale density in the exterior as the number per square centimetre (Murdoch et al. 1989). Here we express scale abundance as the number per twig because the number of scale on a twig is not related to the surface area of the twig. Regressions of the number of scale on stem substrate against stem area, and of the number on leaves against leaf area, from 11 dates, were sometimes significant, but <3.5% of the variance was explained in all cases. Therefore, the abundance of scale on a twig is not affected by twig size. We used number per twig in all analyses, except where we compare scale density in the exterior and refuge (Table 2), in which case we calculated number per unit area.

In the refuge we first mapped and measured the trunk and all branches out to the fourth most recent flush. From 19 June 1984 to 11 March 1985 the refuge was sampled using method 1, in which each sample was selected by (1) choosing a branch (or the trunk) at random (the probability of each unit's being chosen was directly dependent on its length) and (2) choosing

TABLE 2. Differences in average characteristics between exterior and refuge subpopulations of scale. Data were totals for the study period; trees were replicates.

	No. of scale per 100 cm²	Recruitment per female*	Temporal variability†	Percentage parasitism by Aphytis			
				Total hosts	Instar 2	Male instar 2	Instar 3
Exterior							
Mean	1.9	9.56	0.060	19.2	11.5	31.1	28.0
SE	0.3	1.36	0.025	1.0	1.0	2.8	2.2
Refuge							
Mean	189.6	2.22	0	2.3	0.6	2.6	5.4
SE	19.31	0.21	0	0.3	0.2	1.1	0.7

* Recruitment rate is (no. instar 1)/(no. crawler producing females).

† Temporal variability is V_t; see Natural history and methods.

the distance along the branch also at random. Four samples were taken from each tree. Each sample was a 1 cm² disk of bark removed with a cork-borer from the upper surface of the branch. Spatial variance on wood was so high that on 8 April 1985 we changed to method 2, a stratified random design. The branches on all trees were mapped and areas were designated as either high or low density, based on a visual evaluation. We then took two random samples from each density stratum from each tree; overall, scale were ≈3 times more dense on high-density branches.

In all analyses, except Table 2 and the estimation of temporal variability, we use the mean number per disk sample to estimate scale abundance in the refuge. For sampling method 1, abundance in a tree is the average of the four samples but, for method 2, abundance was estimated by weighting the averages from the low- and high-density branches by the fraction each type contributed to the total area of the refuge.

The comparison of scale density in the refuge and the exterior (Table 2) and estimates of temporal variability are based on the number per unit area. The disk samples were taken from the upper surface of the branch where scale are most abundant. The number per square centimetre for a 1 cm wide ring of bark around the branch, Y, was calculated from

$$Y = X^{0.56},$$

where X is the number per square centimetre in the disk sample (Murdoch et al. 1989). Mean number per square centimetre was then estimated as a simple average for method 1 or a weighted average for method 2.

Scale instars 1 and 2 were mistakenly undercounted in exterior samples for the first nine sample dates, of which the first five dates were the pretreatment samples. The undercounting was not biased among trees that would end up in different treatments, so we used the data in tests for effects described in the next subsection. We did not use the data in any analysis that depends

on estimating absolute abundances (e.g., temporal variability) or stage distributions.

Scale are very patchily distributed. We calculated the ratio of sample (i.e., spatial) variance to mean density on each of six dates on which both the refuge and the exterior populations were sampled at random. Scale were more patchy on twigs (median ratio = 9.9, range = 0.8–191.9) than in the refuge (median ratio = 3.7, range = 0.7–27.5).

Parasitism rates in the exterior and refuge were estimated as: (number parasitized hosts)/(number parasitized hosts + number unparasitized hosts). Hosts were often rare, especially in winter. We therefore calculated the fraction parasitized on each date using the total numbers found in all samples and all trees within the treatment on that date. This is a weighted average of within-treatment parasitism on each date.

Adult Aphytis were sampled by taking eight suction samples from the canopy of each sample tree on each date, 1.5 m above the ground, with a Dvac (Dietrich et al. 1959, Dietrich 1961). Two samples were taken per cardinal direction.

For each period between successive sampling dates we calculated the number of degree-days above the threshold for red scale development. Temperature was recorded hourly with a Datapod DP 220 (Omnidata International, Logan, Utah, USA) placed within the canopy of one of the sample trees. Degree-days were calculated from the relationship between scale development rate and temperature developed by Yu and Luck (1988).

Experimental outplants of scale

The standard samples from the exterior and refuge do not provide information on two sources of scale mortality: mutilation of scale by Aphytis and predation by beetles and, perhaps, other predatory insects. Also, in the standard samples we do not know the length of time over which scale in various stages have been exposed to attack. Scale outplanted on lemons provide such information and also parasitism rates on standardized instar 3 scale.

We exposed scale on lemons placed in the exterior of trees on 20 dates. Cohorts of scale were raised to late molt 2 stage on lemons at 25°C in the laboratory. Two-thirds of the lemon surface was covered with wax to prolong the lemon's life. All but 40 scale were removed. Lemons were then hung in the exterior ≈120 cm above ground in the north segment of each sample tree. In each tree two replicate lemons were suspended in nylon wide-mesh bags, thus exposing them to parasitism and predation, and a control was placed in a muslin bag to exclude parasitoids and predators. Lemons were spaced ≈50 cm apart. They were outplanted once a month in June and July 1984 and December 1984 through October 1985, and every 2 wk from August to November 1984.

Lemons were returned to the laboratory after 10 d

in the field. Live scale were instar 3 at the end of the 10-d period except for three dates when a significant fraction of scale completed development to the mature female stage. We combined data from the two lemons exposed to parasitoids and predators on each tree, to give a total of 80 scale per tree on each date. Each scale was scored as live, parasitized by *Aphytis,* mutilated (including host-fed) by *Aphytis,* or preyed upon (i.e., clearly chewed or missing). None of these categories appeared in the control lemons, except for a tiny fraction missing. We therefore analyzed the data directly, without correcting for control observations. Although compensatory mortality may occur (i.e., some that were parasitized might later have been preyed upon), the maximum likelihood estimator of the mortality rate from each mortality class is simply the number dying from a given cause divided by the initial number (A. Stewart-Oaten, *personal communication*).

Statistical tests for treatment effects

We deal first with the standard samples from the refuge and exterior. By chance, trees assigned to the ant-exclusion treatment had fewer scale in the before-treatment period than did those assigned as controls (though the difference was not statistically significant). We therefore tested for the effect of treatment on scale density using as the dependent variable the difference in density in a single tree between the before-treatment period and the after-treatment period. Because estimates of scale density from successive dates are not independent, we used the time-averaged density on each tree. Finally, we are interested in relative changes in density in each tree, independent of the initial density, and we expect changes in density to be multiplicative, reflecting changes in vital rates. We therefore calculated the logarithm of the time-averaged density of scale in each tree in the before period (log N_b) and the logarithm of the time-averaged density in the post-treatment period (log N_a) and asked, using a one-tailed *t* test, whether the difference (log N_a − log N_b) was more negative in ant-exclusion trees than in control trees. Each tree contributes an observation and trees are replicates. The initial undercounting of smaller scale in the exterior should not affect the results, first because counts were not biased among trees that were ultimately assigned to different treatments, and second because we are interested in the effect of treatment on the relative change from pre- to posttreatment abundance in individual trees.

We tested for the effect of treatment on parasitism using again the difference in parasitism in a single tree between the before- and after-treatment periods. Parasitism was often poorly estimated on individual trees in winter when scale were rare. We therefore calculated the time-averaged fraction parasitized in each tree for both the before- and after-treatment periods, by summing the number parasitized, and the number available, over the period and then computing the fraction parasitized. This estimator is thus a weighted average of within-period parasitism rate in each tree.

The estimate of parasitism on each tree in each period in the exterior was arcsine transformed. In the refuge population the fraction parasitized was often <0.1, so we used the logit transformation (Agresti 1990). When parasitism was zero on one or more trees, we added 1 to both the number parasitized and the total number of scale. The presence of a substantial number of zero observations can distort the analysis using the logit transformation because the resulting transformed value depends strongly on the number of observations. Adding 1 to both parasitized and total scale results in a much larger increase in the transformed value for parasitism when there are few observations than when there are many. We therefore always checked the results obtained by this transformation by also using the arcsine transform. The results were qualitatively the same in all cases except one, namely the effect of treatment on the fraction parasitized by *Encarsia* in the refuge.

The "skirt-control" treatment appeared to have had no effect. First, we could discern no differences in the dynamic patterns between scale in control and skirt-control trees, on the basis of simple visual inspection of the data. Second, skirting had no effect on the change in abundance per tree between before- and after-treatment periods in the refuge (*t* test: $t_{14} = 0.09$, $P = 0.93$) or the exterior ($t_{14} = 1.33$, $P = 0.21$), or on the change in fraction parasitized by *Aphytis* (refuge: $t_{14} = 0.28$, $P = 0.78$; exterior: $t_{96} = -0.64$, $P = 0.53$; degrees of freedom reduced since variances unequal) or by *Encarsia* (refuge: $t_{14} = -1.41$, $P = 0.18$, exterior: $t_{14} = -0.53$, $P = 0.60$). We therefore combined the data from these two control treatments to increase sample size (a total of 16 trees distributed in pairs over eight plots in four blocks).

In the outplant experiment there were only two pre-treatment dates so we did not use the pretreatment data in the analyses. Instead, we asked if the posttreatment rates were different in control and ant-exclusion trees. In contrast with the data from standard samples, we treated estimates of mortality rates on different dates as independent observations using a two-way ANOVA with treatment and date as factors. The dependent variable is the logit-transformed mortality rate on each date on each tree; trees are replicates (untransformed data gave the same results). As above, we only considered rates 250 degree-days after the treatment, to allow for the delay in treatment effects. The mean parasitism rate for each of the 16 control trees was based on 1280 exposed scale (80 scale on each of 16 dates).

Estimation of temporal variability

We estimated temporal variability in scale abundance using an index, V_t, that estimates the true variance of successive log densities uncontaminated by spatial variance. A. Stewart-Oaten et al. (*personal communication*) show that standard measures, such as the stan-

dard deviation of the successive logs of mean density, have two problems: (1) they are biased upwards because they are contaminated with spatial variability (estimated temporal variability increases with spatial variance and decreases with sample size), and (2) dates with zero counts are problematic because standard procedures (such as adding a constant before taking logarithms) give measures that are influenced by the (arbitrary) choice of constant (see also McArdle et al. 1990). We replace standard deviation with variance because the latter has better known statistical properties.

A. Stewart-Oaten et al. (*personal communication*) provide a method for removing the bias caused by spatial variance. Spatial (i.e., sample) variance was calculated for each date and then averaged over the study period. The (base 10) log of the average density on each date (corrected for Jensen's inequality, i.e., the log of the mean is not equal to the mean of the logs) was calculated and used to generate total variance in successive log densities. Finally, the true temporal variance, V_t, was obtained by subtracting the mean spatial variance from total variance of log densities. Negative estimates can occur and are set equal to zero.

Dates with zero counts present severe problems (A. Stewart-Oaten et al., *personal communication*) and occurred when we used trees as replicate observations. We therefore removed most zeroes by combining samples from the two trees in each of the 12 plots. A few zeroes remained and were treated in the standard way by adding a constant to the mean density from each pair of trees on all dates, before taking the log. There are no well-justified rules for choosing the constant, and different values lead to different estimates of temporal variability. We followed Mosteller and Tukey (1977) and added $\approx 1/6$ of the smallest observation. In practice our choice of constants did not affect the results (the difference in temporal variability between treatments) because the answer remained the same when we repeated the calculations with a range of constants up to the smallest observation. The sampling unit for temporal variability is therefore two trees that were close together but widely separated from other trees in the grove. The estimate of spatial variance on each date came from the individual samples (a total of four or eight on a date, depending on the date and substrate).

Tests for density dependence

To test for temporal density dependence in scale mortality from various sources, we analyzed data from both the standard samples from the 16 control trees and from outplanted lemons on these same trees. In all cases, the dependent variable was the arcsine- or logit-transformed mean rate (of parasitism, host mutilation, or predation) on a given date estimated across all the samples or lemons available on that date. The dependent variable was the mean density of scale on a date estimated across all samples from the 16 control trees.

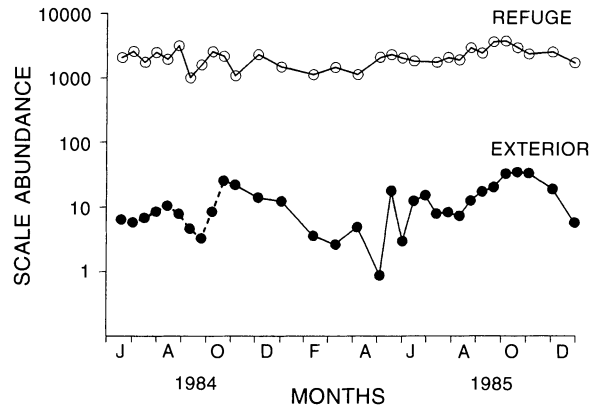We regressed the transformed fraction parasitized in

Fig. 1. Number of scale per 400 cm² of surface area (the average size of a twig) in the exterior and refuge sections of the tree, plotted on a log scale against calendar date. Exterior abundance was undercounted on the first nine dates (dashed line; see *Natural history and methods*).

standard samples against host density, for total hosts (i.e., instar-2 + instar-3 females + instar-2 males) and for instar-3 females only since this is a preferred stage and was also the stage available on outplants. The independent variable was current host abundance, or host abundance lagged by one or two sampling dates. To analyze the data from outplanted instar 3 we regressed the rates of parasitism, host mutilation, and predation against the density of available hosts or prey in standard exterior samples, present either concurrently or on the previous sampling date (i.e., lag = 1).

RESULTS

Differences between refuge and exterior populations in control trees

The refuge hypothesis states that stability of the exterior population results from a relatively constant influx of crawlers from the refuge in the tree's interior. Before testing the hypothesis we first confirmed that parasitism was lower in the interior, and that scale density was ≈ 100-fold higher in the interior even though recruitment per female was lower there (Table 2).

Second, a relatively constant production rate of crawlers in the interior would be more likely if the population of scale in the refuge fluctuated less than did that in the exterior. Scale density in the refuge was significantly more constant ($V_t = 0$; we did not detect any temporal variation) than that in the exterior (mean $\pm$ 1 SE = 0.06 $\pm$ 0.025) (paired t test; $t_7 = 2.4$, $P = 0.048$, Table 2). Our estimate of $V_t = 0$ in the refuge implies that the apparent temporal variability seen in the refuge population (Fig. 1) cannot be distinguished from sampling error.

The larger fluctuations in the exterior population were associated with more discrete cohorts there. The difference is illustrated by abundances of scale stages 1, 2, and 3 in the two parts of the tree (Fig. 2) and
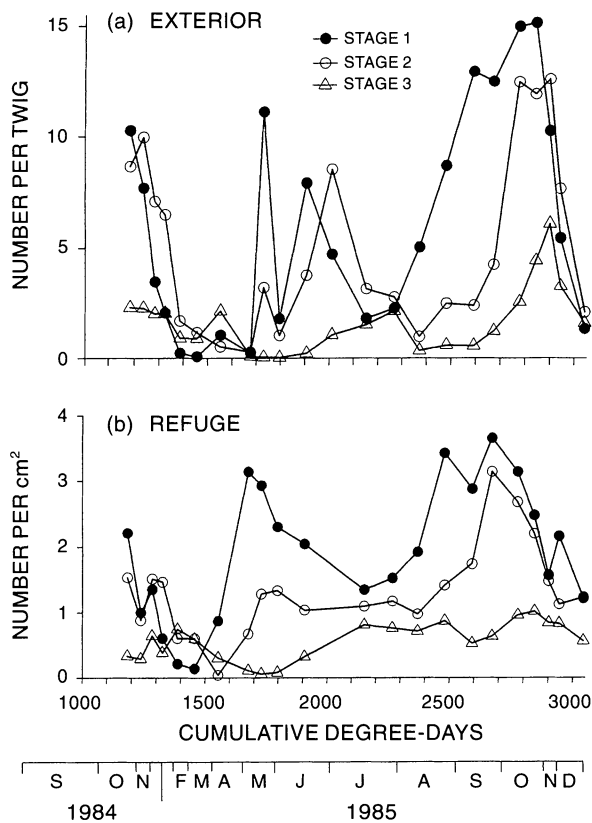
FIG. 2. Number per sample of stage 1 scale (instar 1 + molt 1), stage 2 (instar 2 + molt 2) and stage 3 (instar 3 + mature females) in (a) exterior and (b) refuge sections of the tree, plotted against the number of degree-days accumulated since the start of the study. Calendar dates are also shown. Counts are averages over the 16 control trees. Data start just after degree-day 1000 because smaller stages were undercounted in the exterior early in the experiment.
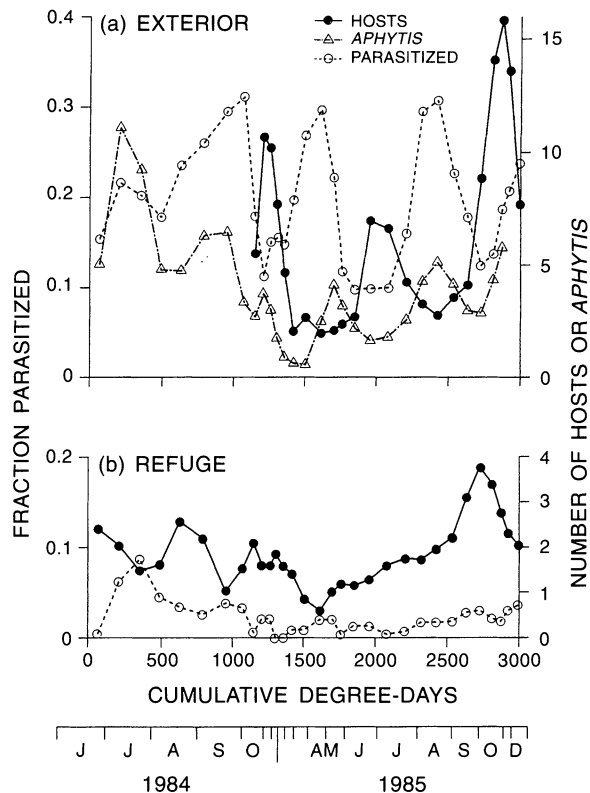

FIG. 3. Two-date running averages of the number of total hosts (instar 2 + instar 3 + male instar 2) per sample and fraction of total hosts parasitized by *Aphytis* in (a) the exterior and (b) the refuge regions of the tree. Panel (a) also shows 1.5× the number of female *Aphytis* in Dvac samples. Counts are averages over the 16 control trees. Estimates of host abundance in the exterior prior to degree-day 1000 are not shown because the smaller stages were undercounted.

especially by the abundance of host stages and the parasitism rate (Fig. 3). We cannot determine whether the discrete cohorts were caused by seasonal effects on scale phenology and demography, including synchronizing effects of winter, or whether parasitism also played a role. A pulse of hosts is followed after ≈450 degree-days by a burst of flying adult female *Aphytis* (Fig. 3a).

Effects of ant exclusion

The reduction in ants was expected to increase parasitism in the refuge, to reduce scale density in the refuge, to reduce export of crawlers to the exterior, and hence to reduce stability of the exterior scale population. We next test each prediction in turn.

Effects on parasitism, host mutilation, and predation.—Parasitism of scale by *Aphytis* in standard samples increased in the refuge in trees where ants had been excluded, relative to control trees (Fig. 4b). The difference disappeared on three dates near the end of the experiment, but was significant when averaged over

the whole posttreatment period ($t_{22} = 2.96$, $P = 0.003$, one-tailed test). Parasitism rate in the posttreatment period in ant-exclusion trees was 0.06 ± 0.01 (mean $\pm$ 1 SE) (double the pretreatment rate in ant-exclusion trees) vs. 0.02 ± 0.003 in control trees (about half the pretreatment rate in these trees). Even after ants were excluded, however, parasitism in the refuge was still only ≈1/3 of the level in the exterior (0.20 ± 0.02).

Excluding ants did not affect parasitism rate in standard samples from the exterior of trees (Fig. 4a, $t_{22} = 1.02$, $P = 0.32$). This result is consistent with the observation in the exterior of no difference between treatments in the change in the number of female *Aphytis* in Dvac samples (taken in the exterior of trees) from the pre- to posttreatment period ($t_{22} = 1.27$, $P = 0.22$). Ants were rarely seen in the exterior of trees, which may explain why ant exclusion increased parasitism in the refuge but not in the exterior.

Excluding ants also did not affect the rates of parasitism, host-mutilation or predation of scale on lemons outplanted in the exterior of trees. In all cases there was a significant treatment × date interaction, a sig-
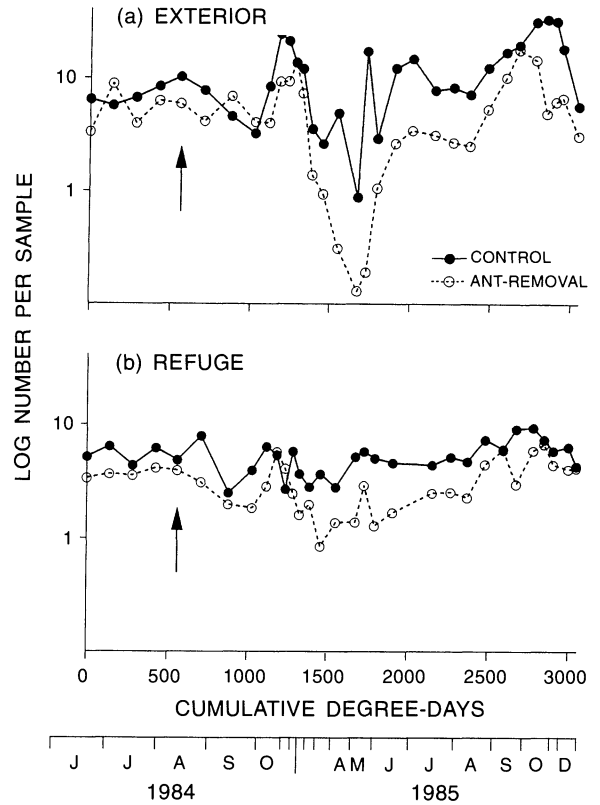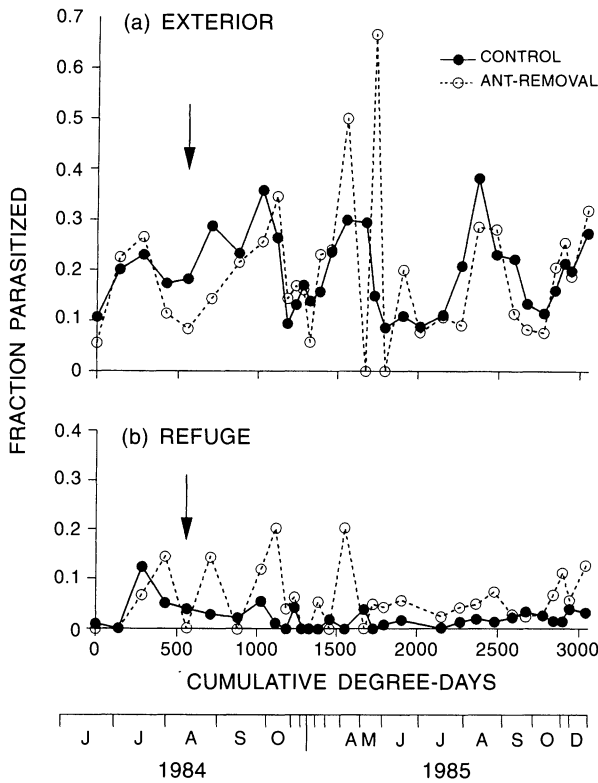
FIG. 4. Fraction of total hosts parasitized by *Aphytis* in (a) the exterior and (b) refuge regions of control and ant-removal trees both before and after the ant-exclusion treatment was carried out. The arrows indicate the date on which the treatment was done.

FIG. 5. Logarithm of mean number of total live scale per sample in (a) the exterior and (b) the refuge regions of control and ant-removal trees both before and after the treatment was carried out. The arrows indicate the date on which the treatment was done. Small stages of scale were underestimated on the first nine samples in the exterior.

nificant effect of date (reflecting seasonal changes in these mortality rates), but no main effect of ant exclusion (Table 3). Again, the absence of an effect of ant exclusion may reflect the scarcity of ants in the exterior of control trees.

Ant exclusion had no effect on parasitism by the parasitoid, *Encarsia,* as measured in standard samples from the exterior ($t_{22} = -0.16$, $P = 0.87$). Using the arcsine transformation, there was also no effect on *Encarsia* parasitism in the refuge ($t_{22} = 0.61$, $P = 0.55$). There was a significant decrease in fraction parasitized based on the logit transformation. However, 35% of the values for parasitism were zeros. A plot of the data shows the significant effect was entirely owing to these

zero observations, and in this case we accept the analysis based on the arcsine transform.

Effects on scale density in the refuge.—The difference in scale density in the refuge between control and ant-exclusion trees increased as a result of a decline in scale density in ant-exclusion trees (Fig. 5b). The effect was statistically significant averaged over the whole posttreatment period ($t_{22} = 2.05$, $P = 0.027$; one-tailed test). However, posthoc tests applied separately to the data in the early posttreatment period (between degree-days 1180 and 2480; $t_{22} = 2.56$, $P = 0.009$), and from there to the end of the experiment ($t_{22} = 1.48$, $P = 0.08$) confirm the impression (Fig. 5b) that the effect

TABLE 3. Two-factor analysis of variance on effects of treatment (control vs. ant exclusion) and date on parasitism, host mutilation, and predation. Fractions were logit transformed. ** $P < 0.01$, *** $P < 0.001$.

Source of variation	df	Parasitism		Host mutilation		Predation	
		MS	F	MS	F	MS	F
Treatment	1	0.97	0.38	3.87	2.21	4.28	1.71
Date	15	35.56	39.51***	11.41	13.91***	12.02	16.93***
Treatment × Date	15	2.51	2.79***	1.75	2.13**	2.51	3.54***
Error	351	0.90		0.82		0.71	

on scale density in the refuge disappeared at the end of the experiment. Over the early posttreatment period average density in the refuge in treatment trees was only 50% of that in the control trees compared with 70% in the pretreatment period.

Effects on scale density in the exterior.—In contrast to the effect in the refuge, the change in the difference between control and ant-exclusion trees was significant not only over the whole posttreatment period ($t_{22} = 2.38$, $P = 0.014$, one-tailed test) and in the earlier part of that period ($t_{22} = 1.90$, $P = 0.035$, one-tailed test), but also in the latter half of the experiment ($t_{22} = 2.20$, $P = 0.019$, one-tailed test). Scale density in the exterior, averaged over the entire posttreatment period (degree-days 1180–3040), was 39% of control compared with 76% during the pretreatment period. For the last few months of the experiment, however, scale density in the exterior returned to the range seen at the start (Fig. 5a).

The fact that scale density in the exterior was strongly reduced by ant exclusion, even though *Aphytis* parasitism rates in the exterior were not increased while those in the refuge were, suggests that the reduction in scale density in the exterior resulted indirectly from the reduction of scale density in the interior, rather than from direct effects of ants on parasitism rate in the exterior.

Effects on temporal variability.—We compared the temporal variability of the exterior population in the control trees to that in the ant-exclusion trees. As predicted by the hypothesis, temporal variability in the exterior scale population during the posttreatment period was higher in ant-exclusion trees ($V_t = 0.28 \pm 0.048$ [mean $\pm$ 1 SE]) than in control trees ($V_t = 0.06 \pm 0.025$ [mean $\pm$ 1 SE]) ($t_{10} = 4.5$, $P = 0.002$, one-tailed test). Increased variability appears to have been caused by a marked decrease in density in the ant-exclusion trees in winter 1984–1985, followed by a tendency to increase towards the control density at the end of the experiment (Fig. 5a). However, over the last few months of the experiment variability in the ant-exclusion trees appeared to be no greater than in the control trees (Fig. 5a). Unfortunately, the experiment ended when the grove was sprayed in December 1985, and we cannot tell whether the treatment effect had disappeared or if variability would have increased again in the following year.

There was no effect of treatment on temporal variability in the refuge. Mean temporal variability in the four ant-exclusion plots was low ($V_t = 0.05 \pm 0.05$ [mean $\pm$ 1 SE]; in three of the plots $V_t = 0$), and not statistically significantly different from zero. There was also no detectable temporal variation in the refuge population in control trees ($V_t = 0$ for all plots).

Possible sources of density dependence

We used the data from samples and outplanted scale in control trees to test for density-dependent relationships that might contribute to stability of the red scale/*Aphytis* interaction.

Parasitism and host mutilation by Aphytis, *and predation, in relation to scale density.*—The rate of parasitism on a given date in the exterior of control trees was not positively related to either current or previous scale density. None of the six regressions of arcsine-transformed parasitism rate against exterior scale density (total hosts and instar 3, each with a lag of 0, 1, and 2) was significant (all $P > 0.1$); all r^2 values were ≤ 0.1.

In the refuge, parasitism by *Aphytis* was low, relatively invariant, and not obviously related to the abundance of host scale stages (Fig. 3b). Only one of the six regressions of logit-transformed fraction parasitized against host density was statistically significant (total hosts, lag = 2, $P = 0.01$, $r^2 = 0.23$) and the relationship was positive.

There is a conceptual difficulty in looking at delayed density dependence in populations with overlapping generations and pulses of hosts: it is possible to detect spurious density dependence. Even if instantaneous parasitism rate per parasitoid is fixed, pulses of hosts will likely be followed by pulses of parasitoids, and hence by out-of-phase pulses of percent parasitism. This occurs, for example, in the neutrally stable density-independent Lotka-Volterra model. Thus, we might detect density dependence in the parasitism rate if we were to plot it against host density ≈ 375 degree-days earlier. We did not do this posthoc analysis.

Parasitism rates from outplants also showed no direct density dependence. We regressed the logit-transformed rates of parasitism, host mutilation, and predation on instar 3 that had been outplanted on lemons in control trees against the density of available hosts or prey in standard twig samples present either concurrently or on the previous sampling date (i.e., lag = 1). There were no significant positive regressions, regardless of whether "hosts" or "prey" were defined as instar 3 available, or as all possible hosts or prey. However, the regressions of parasitism rate against the densities of both current and lagged instar 3 and total hosts were significantly inversely density dependent (instar 3: unlagged, $P = 0.06$, $r^2 = 0.19$; lag = 1, $P = 0.008$, $r^2 = 0.35$; total hosts: unlagged, $P = 0.02$, $r^2 = 0.25$; lag = 1, $P = 0.002$, $r^2 = 0.45$).

The average fraction of outplanted instar 3 that were mutilated (0.056) was about half that parasitized (0.094) and preyed upon (0.096). The similarity of the parasitism and predation rates suggests that predation might have a substantial effect on scale dynamics. However, predation of instar 3 on lemons may not reflect rates on the scale population in trees.

Encarsia dynamics.—Parasitism by *Encarsia* in the exterior (Fig. 6a) was lower than that by *Aphytis* (Fig. 3a), and was again much lower in the refuge (Fig. 6b) than in the exterior. There are no clear oscillations in parasitism rate by *Encarsia*.

As in the case of *Aphytis,* there is no obvious relationship between parasitism rate and host density. We regressed the fraction of hosts parasitized against host density, using a lag of 0, 1 and 2 sampling dates, for both the exterior and refuge populations. None of the regressions of fraction parasitized was significant.

Fluctuations in the rate of parasitism by *Encarsia* were uncorrelated with the fluctuations in the rate of parasitism by *Aphytis,* both in the exterior ($r = -0.056$, $P = 0.80$) and the refuge ($r = 0.30$, $P = 0.10$).

Discussion

Stability and the refuge hypothesis

The connection between stability and amount of temporal variability in population density is not necessarily simple (e.g., Horwood 1993, Taylor 1993). Nevertheless, we hypothesize that the observed constancy in red scale populations must reflect, in part, the existence of stabilizing processes that regulate around a relatively invariant equilibrium (Murdoch 1994).

Removal of the refuge might have destabilized the scale population in either of two ways. First, it might have induced limit cycles, or increased the amplitude of any existing cycle. Either effect ought to be detectable by an increase in temporal variability in population density. In either case, though, the population would have remained regulated, i.e., bounded (Murdoch 1994). Second, removal of the refuge might have caused the population to become unregulated. In our study this should have caused a trend in density towards zero in the ant-exclusion trees where the refuge population was reduced.

The results of ant exclusion were equivocal. We confirmed that the interior is a partial refuge from parasitism and that the refuge population fluctuated little, which are prerequisites for the refuge hypothesis. Removal of ants increased parasitism in the refuge by *Aphytis* and reduced scale density in the refuge. Finally, the predicted increase in the size of fluctuations in density occurred in the exterior. However, by the end of the experiment, temporal variability in the exterior appeared to be about the same in ant-exclusion and control trees (Fig. 5a), and only a longer experiment could tell us whether the increased temporal variability was temporary.

The scale population in the exterior in the ant-exclusion trees did not drift towards zero (Fig. 5a), so ant exclusion did not result in loss of regulation. The experiment lasted long enough (16 mo) for a large decline in scale density to occur. For example, DeBach (1958) found that scale density in one population increased 20-fold in a single year when control by *Aphytis* was interrupted and, in an adjacent plot, decreased 15-fold when control was regained.

The experiment may not have provided a critical test of the hypothesis. Removing the ants resulted in a relatively small increase in parasitism in the refuge and
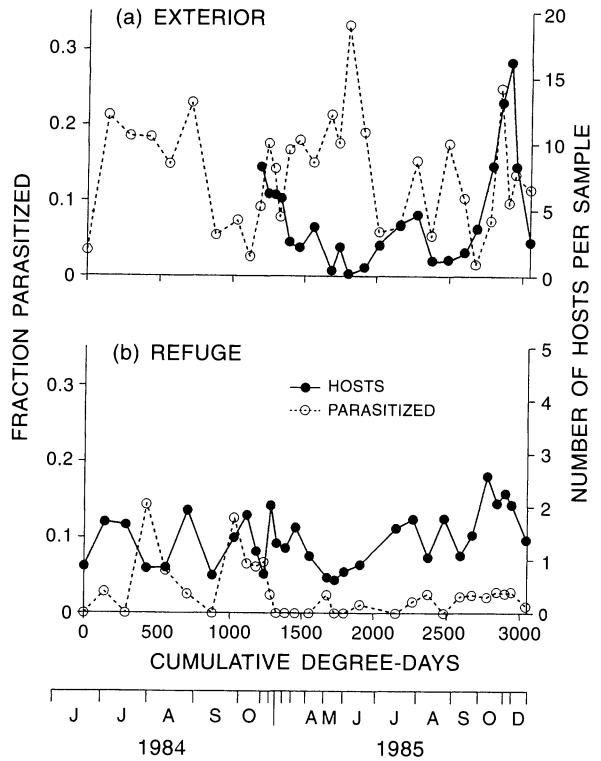

FIG. 6. Number of hosts per sample and fraction parasitized by *Encarsia* in (a) the exterior and (b) refuge portions of the 16 control trees. Host abundance in the exterior is not shown prior to degree-day 1000 because smaller host stages were undercounted.

a relatively small decrease in scale density in the refuge. Parasitism in the refuge of treated trees was still only 32% of that in the exterior, and scale in the refuge of treated trees were still ≈100 times denser than in the exterior. Thus, we did not achieve the crucial test of removing or drastically reducing the refuge population.

Constancy of the scale population

The scale population was remarkably constant in density compared with other populations, especially considering it is a pest insect, albeit now under control. The standard deviation of the (base 10) logarithm of successive scale densities (henceforth SD logs) of the total scale population was only 0.12. This value is at the very low end of the range found in natural and other populations. For example, only 5% (4/76) of populations recorded by Connell and Sousa (1983) that had been sampled at least 5 times had lower SD log values. Similarly, among 16 insect species associated with goldenrod that were sampled in at least 4 yr, in 21 different habitats (giving a total of 336 populations), only 4 (1%) showed an SD log ≤ 0.12 (Root and Cappuccino 1992, Fig. 5). The SD log for the total scale population was also lower than the values for all but 15 of the 91 syrphid species collected over 15 yr by

Gilbert and Owen (1990). These 15 were all extremely rare species, and Gilbert and Owen (1990) added 1 to counts before log-transforming, which causes the SD log value to be artificially low in rare species.

There are problems with the above comparisons. First, the SD log measure is contaminated by spatial variance (A. Stewart-Oaten et al., *personal communication*), which varies from one study to another. Second, unlike our study, many others underestimated temporal variability by adding 1 to zero counts. Third, the sources of variation are different in the two sets of populations. Variation in our scale population was measured over 23 dates, and three generations. By contrast, there is typically no within-generation variation in the studies of annual species (which, all else equal, should therefore be less variable).

We have not underestimated the long-term variability of scale populations under control by *Aphytis*. P. DeBach (*unpublished data* held by R. Luck) took exterior samples each month between 1962 and 1971 from a grove in southern California. The SD of the successive log abundances is only 0.36, almost the same value as in our study (0.4).

Stabilizing mechanisms

There is strong historical evidence that *Aphytis* drastically suppressed scale from its original outbreak levels, and maintains it at low stable densities (DeBach et al. 1971, Rosen and DeBach 1979). The refuge population is especially stable, but we do not know what stabilizes it. Murdoch et al. (1989) removed the refuge population in several trees and within 6 mo densities were indistinguishable from those in control trees, suggesting strong negative feedback. Density in the refuge may often be near a resource limit at places where dense scale overlap each other. However, there is also extensive bark area with few or no scales.

An alternative to the refuge explanation is that the stabilizing mechanisms lie in the parasitoid–host interaction itself, such as in the size-structured interactions (Murdoch et al. 1992, Murdoch 1994), but we have found here little empirical evidence for this view. Parasitism, host mutilation, and predation were not temporally density dependent, even when time lags were incorporated into the analyses. Instead, there was evidence for inverse density dependence.

Our failure to detect density-dependent mortality on scale may result because any density dependence is obscured by time lags and seasonal effects. Such complexities are largely absent in the much more commonly studied univoltine insects. In addition, it is statistically difficult to detect density dependence when random effects on density are combined with only small departures in density from equilibrium.

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ERRATUM

In an article by M. Mangel and F. R. Adler ("Construction of multidimensional clustered patterns," *Ecology* **75**(5):1289–1298), on p. 1292, in Step 3 of *"Algorithm 1: constructing the structure function from a pattern,"* both the numerator and the denominator should be summed over x_2 instead of x_1, and X_2 should be x_2 at the end of the expression on the right-hand side. Thus, Step 3 should read:

$$p_{x_1}(r|1) = \sum_{x_2} Z(x_1)Z(x_2)I[r, d(x_1, x_2)] \Big/ \sum_{x_2} I[r, d(x_1, x_2)].$$