

Population Structure and the Conundrum of Local Adaptation

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The black pineleaf scale insect (*Nuculaspis californica* Coleman) is a parasite of western yellow pine (*Pinus ponderosa* Lawson) and 11 other conifer species (Ferris 1938, Furniss and Carolin 1977). The insects are short-lived relative to their host trees, largely sedentary, and achieve persistent, damaging infestations in areas where airborne dust or the drift of orchard insecticide compromises biological control agents. The abundance of black pineleaf scale varies within an infested stand and correlates with the age and size of the trees; larger, older pines harbor more scales than smaller, younger ones. In the same paper that laid out this basic biology, George Edmunds (1973) was first to suggest that “scale populations apparently become adapted to specific host individuals, and population densities can become high only with genetic fitness of the population to the host species and individual.”

I began graduate work under Edmunds’ tutelage just as the 1973 paper was published, shared his interest in local adaptation, and worked sporadically with this system for nearly twenty years, both in collaboration with George, and after his retirement. In this chapter I provide a retrospective of that work, beginning with background on the scales’ natural history and basic hypotheses pertaining to population structure; summarizing three areas of empirical research including transfer experiments, analyses of scale population biology, and allozyme studies of population genetic structure; and closing with my interpretation of these results and their implications.

Background

At field sites in the northwestern United States, black pineleaf scale insects complete one generation per year. Beginning in early July after new pine needles become fully elongated, individual females lay up to 70 eggs over a three-week period. Eggs hatch quickly, and teneral larvae harden beneath the maternal scale cover before walking to feeding positions on new needles. Young scales are vulnerable to desiccation, and most settle on the same twig that bore their mothers. Nevertheless, larvae of both sexes may be passively dispersed by the wind during this "crawler" stage, and colonies are founded on previously uninfested host trees by wind-blown females (Edmunds 1973, Edmunds & Alstad 1981, Alstad & Edmunds 1983b).

After inserting their mouthparts through a stomate into mesophyll cells, larvae secrete a protective scale cover which is firmly attached to the pine needle, and molt to a legless second instar. Females never move again after this initial larval settlement (Baranyovits 1953, Beardsley and Gonzalez 1975, Miller & Kosztarab 1979). Scales feed through fall and pass a winter diapause *in situ* before reaching the third instar in early spring. Males are sedentary until mid-May when they metamorphose and crawl from beneath scale covers to mate with females on the

same and nearby pine needles (Alstad et al. 1980). The winged males may also fly between trees, providing a second vehicle for gene flow beyond the natal host.

Like the olive scale (*Parlatoria oleae* Culvee) on California citrus crops (Murdoch et al. 1984) and the pine needle scale (*Chionaspis pinifoliae* Fitch) that infests numerous conifer species (Luck 1973; Luck & Dahlsten 1974, 1975), the black pineleaf scale has important natural enemies, and damaging scale outbreaks occur where these biological control agents are compromised or absent. *Coccinellid* predation affects the dynamics of *Nuculaspis*, but the principal determinant of its abundance is the *aphelinid* parasitoid *Coccobius varicornis* (Howard) (J. Wooley, personal communication; Hayat 1983). Our major study sites are all in areas affected by dust from unpaved roads, or insecticide drift from orchards, both of which reduce the wasp's effectiveness and allow variations in scale insect density.

Hypotheses

Tree-to-tree differentiation and genetic structure in a scale insect population could have several potential causes. Natural selection might increase the local frequency of insect traits adapted to individual trees (a Local-Adaptation Hypothesis). Local adaptation hypotheses are further divisible according to the nature of the host-plant characteristics which mediate the selective process. At one extreme, insects might become adapted to intrinsic host traits that are under strong genotypic control. Alternatively, if environmental influences are relatively constant from generation to generation, insects might adapt to extrinsic features of the phenotype that may be entirely of environmental origin, or the product of interactions between the tree's genotype and environmental circumstances; for example, scales might become adapted to trees that are in especially good or bad condition with respect to nutrient availability, water relations, or the stress imposed by pathogens or herbivores such as scale insects themselves. These mechanistic alternatives distinguish two local-adaptation hypotheses that I will call the Intrinsic and Extrinsic Local-Adaptation Hypotheses. The essential difference between them is the spatial distribution of plant traits that cause the adaptive response. The Intrinsic Hypothesis assumes that all foliage with the same genotype (i.e., most of the tree with the apical dominance of conifers) provides a homogeneous selection regime, while the Extrinsic Hypothesis does not.

As an alternative to both local-adaptation hypotheses, it is also possible that the sedentary habit of scale insects might allow variations to arise by chance (a Drift Hypothesis). This implies that the limited gene flow resulting from movements of larvae and winged males is insufficient to homogenize spatial variations in allelic frequency that arise as sampling effects. The limited number of insects on individual branches, their extreme polygyny (see below), and their haplodiploid inheritance will all foster sampling effects; thus the basic natural history of black pineleaf scale suggests that drift may be important.

Transfer Experiments

We began empirical work on the pine/scale interaction with an adaptationist perspective. We were especially drawn to the Intrinsic Hypothesis because trees that produce recombinant progeny might be less likely to pass preadapted pests to their offspring, and parasites such as the black pineleaf scale might therefore drive the obligate sexuality of pines (Williams 1975;

Maynard Smith 1978; Linhart et al. 1979, 1981; Hamilton 1982; Rice 1983; Herre 1985; Lively 1987; Seger & Hamilton 1988; Michod & Levin 1988; Frank 1993). In this context, the logical first step was a series of transfer experiments, moving scale insects within and between individual host trees and quantifying their survival.

Our first such experiment involved the transfer of insects from 10 infested trees near Spokane, Washington to 10 uninfested trees, with three replicates in each of 100 combinations. The resulting ANOVA showed significant differences in insect survivorship attributable to (a) the donor trees that served as sources of transferred insects, (b) the receptor trees (to which insects were moved) and (c) donor * receptor interaction. We interpreted these effects as evidence of local adaptation to intrinsic traits of individual pines (Edmunds & Alstad 1978). This experiment and its weaknesses became fairly well known (*cf.* Unruh & Luck 1987, Hairston 1989). In particular, we were concerned about moving insects that had caused persistent damage in the Spokane area, and hence chose receptor trees 30 km away in Deer Park, where we knew that weather would gradually snuff out the aftermath of our experiment. A more restricted test of the hypothesis that insects are adapted to individual host trees is a reciprocal transfer, where the same trees serve both as the source and destination of manipulated insects, eliminating locality as a source of performance variation.

Following the work presented in the 1978 paper we developed techniques for removing resident scales from individual branches intended to receive experimental transfers, improved our procedure for manipulating the samples, strengthened both quantitative detail and the range of response parameters, and employed these improved methods in three fully reciprocal transfer experiments at three different field sites. The example I present here is a 1988-9 experiment performed at Dryden, Washington, within a five-hectare area adjacent to orchards in the Wenatchee River Valley. We moved insects within and between five host trees in all combinations. Scale-infested pines were chosen with wide variation in physical condition, as evidenced by needle elongation and retention, to maximize the probability of detecting local

Table 1. Average survival of scale insects among replicate transfers in 25 pairwise combinations. Values represent the ratio of survivors to initial colonists. Within-tree survival values are on the diagonal.

DONOR	RECEPTOR				
	615	634	651	652	653
615	0.506	0.329	0.548	0.304	0.856
634	0.637	0.742	0.493	0.568	0.624
651	0.444	0.845	0.290	0.455	0.269
652	0.532	0.642	0.266	0.416	0.301
653	0.374	0.677	0.333	0.424	0.455

adaptation associated with host vigor. The experiment was designed for a two-way analysis of variance and there were four replicate transfers in each donor-receptor combination. Sixteen samples were lost to tip moths, scarabaeid grazing and other causes. Transfer twigs were moved in mid-July of 1988, 11,400 initial colonists were counted in place on marked needles in early

August, and surviving scales were harvested for analysis in May, 1989. The matrix of average survival values for different donor * receptor combinations (Table 1) shows no indication of significant differences in the survival of insects that were moved within (on the diagonal) and between (off of the diagonal) trees.

ANOVA of the ratio of surviving insects to initial colonists (Table 2) demonstrated a marginally significant receptor effect (some trees were more difficult to colonize than others), but neither the donor treatment nor the donor * receptor interaction contributed significantly to survival patterns.

Table 2. ANOVA table for the reciprocal transfer experiment, calculated on the ratio of survivors to initial colonists after angular transformation. The treatments are Donor Tree Identity and Receptor Tree Identity.

SOURCE	DF	SS	MS	F	P
DONOR	4	0.425	0.106	1.34	0.264
RECEPTOR	4	0.775	0.194	2.45	0.055
DONOR * RECEPTOR	16	1.686	0.105	1.33	0.208
ERROR	59	4.666	0.079		
TOTAL	83	7.552			

A similar analysis of surviving sex ratios (rationale for use of the late-instar sex ratio as an experimental response parameter is given in the next section) yielded the same result with more statistical confidence; the receptor treatment contributed significant variance with $p < 0.02$. Again, neither the donor treatment nor the donor * receptor interaction was significant. In a step-wise analysis, the strongest predictor of insect survival on individual pine needles was the density of initial colonists; scales suffered higher mortality on needles that received a high-density inoculation.

The survival data in this fully reciprocal design were different from those we reported for the 1978 experiment, and inconsistent with the hypothesis that scales were adapted to intrinsic genotypic characteristics of individual trees. The fact that this experiment has gone unpublished to date reflects my struggle to make sense of the two results. In addition to genotype, there were undoubtedly many extrinsic factors that varied between the trees at Spokane and Deer Park, and any between-tree transfer design that is executed without a common-garden arrangement controlling environmental variables (including the one reported by Edmunds & Alstad 1978) will confound intrinsic and extrinsic attributes of the host plants.

Scale Insect Population Biology

If insects become locally adapted, then survival and reproductive success ought to index their relative adaptation. To explore this possibility, we began keeping annual records of scale density and sex ratios on individually numbered trees from study sites near Spokane and Dryden, Washington, and The Dalles, Oregon. We chose trees with a range of insect densities, presuming that this sample would reflect differing levels of local adaptation.

These census data showed wide variation in the sex ratio measured just before male eclosion (when they fly and can no longer be counted). Tree-to-tree variation in the proportion of males ranged from 1% to 6% in 1984 (Figure 1) and 1% to 30% in other years (Figure 3). In addition, the proportion of males on a tree was correlated with density; trees with many scale insects had a higher proportion of males (Alstad & Edmunds 1983a).

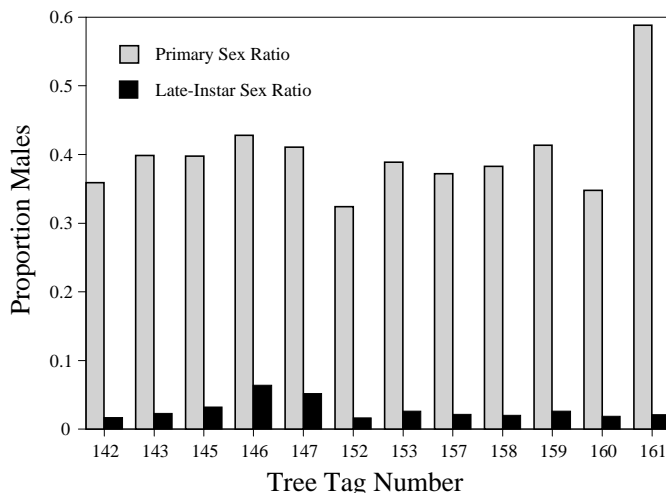


Figure 1. Primary and late-instar sex ratios. Primary ratios showed a higher male frequency than late-instar ratios from the same generation. Samples were collected at Spokane in July 1983 and May 1984. Redrawn after Alstad & Edmunds 1989.

Sex ratios might vary locally through differential dispersal of the sexes, differences in primary allocation to sons and daughters, or differential survival. To test the dispersal hypothesis we counted early-instar sex ratios just after larval settlement. These data were comparable to the primary ratios in Figure 1, showing little difference in the dispersal of males and females (Alstad & Edmunds 1989). None of the many optimality models of primary sex allocation predict a sex ratio lower than 25% sons in a multiple-foundress system (Hamilton 1967, Bulmer & Taylor 1980, Taylor & Bulmer 1980, Werren 1980, Wilson & Colwell 1981, Charnov 1982, Nunney 1985), so we presumed that much of the variation we were observing was due to survival differences. After learning to sex first-instar larvae (by their sensillae and setal pattern, Stoetzel & Davidson 1974), we confirmed that primary sex allocation is modestly female biased, varying little from tree to tree and from year to year, and ranging among trees from 35% to 45% sons (Alstad & Edmunds 1989). Thus most of the tree-to-tree sex ratio variation we observed late in the insects' life cycle was attributable to differential survival of males and females in the interval between settlement and mating. Differences in mortality following treatment with the insecticide Malathion also cause the surviving sex ratio to become increasingly female biased (Edmunds & Alstad 1985). We interpret these within-generation changes in the sex ratio as evidence of selection, revealed by a method comparable to the "cohort analyses" of Endler (1986).

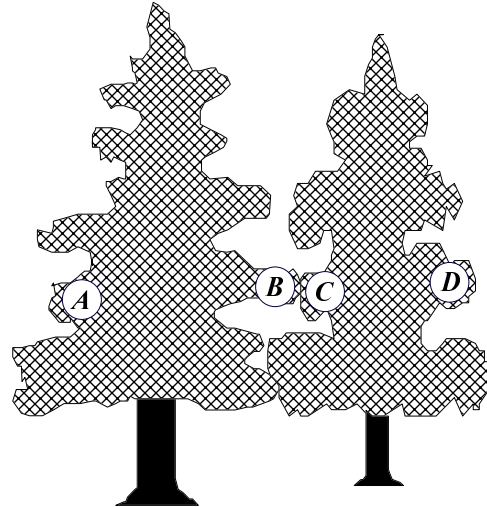
Scale insects in the family *Diaspididae* are haplodiploid. Development of both sexes is initiated by obligate fertilization, but the paternal chromosomes of males then become dysfunctional, so that sons are haploid and hemizygous at all loci. Daughters express both maternal and paternal components of their diploid genotype (Bennett & Brown 1958; Brown 1958, 1965; Brown & McKenzie 1962; Nur 1967, 1971; Bull 1983). Because the insects are completely sedentary in the interval between larval settlement and mating, variations in the survival of males and females can be associated with both the local selective regime and genetic attributes of the scales (sex and ploidy). In particular, any polymorphic locus with a dominant, locally adaptive allele will express the adaptive phenotype at frequency p in males and $2pq+p^2$ in females. With maximum genetic variance at $p = q = 0.5$, half again as many females as males are expected to express that adaptive character. This female advantage will fall and then disappear as selection raises the frequency of locally adaptive traits to fixation. This simple genetic model suggests that the late-

instar sex ratio (which is a correlate of insect density) is also a correlate of genetic variance and local adaptation (Alstad & Edmunds 1983a, 1989). It predicts that relative male survival and the late sex ratio will rise as genetic variance falls under selection, and local adaptation of the insect deme increases.

We tested the hypothesis that late-instar sex ratios vary inversely with genetic variance by measuring late sex ratios on the adjacent and isolated sides of paired trees standing in close proximity. We chose tree pairs whose limbs touched and interlaced on one side, but were separated on the other side by at least five meters from the foliage of any other tree (Figure 2). We reasoned that the limited movement of scale insects would cause more gene flow in the contact zone where foliage of two different trees touched than on their isolated sides. Gene flow should lead to the introgression of maladaptive alleles from the adjacent tree, increase genetic variance, and reduce the survival of haploid male scales relative to diploid females. Nineteen of 22 late-instar sex ratio comparisons showed a greater female bias on the adjacent side relative to the isolated side of the same tree. Scale insect densities were also lower near the contact zone. We interpreted these data as evidence of an outbreeding depression in the contact zone between adjacent demes (Alstad & Edmunds 1983a, 1987). In retrospect, two possible mechanisms could produce this result.

The outbreeding interpretation assumes that selection is homogeneous across the tree, and insects vary as a result of near-side introgression of maladaptive alleles. It is also possible that insects are panmictic across the tree, and selection imposed by differences in the pine foliage varies from one side to the other. There is variation across the foliage of an individual pine in carbon/nitrogen ratios, terpenoid composition and concentration, and the number and extent of resin ducts in needles (Johnson, Young and Alstad, unpublished); it is possible that these traits are consistently distributed with respect to our near-side, far-side dichotomy, biasing the sex ratio without causing differentiation to persist beyond the current generation (Jaenike 1981).

Just as scale survival and density vary from tree to tree, they also vary from year to year. The relative survival of males was correlated with total scale density on individual trees, as seen for two years of different average density in Figure 3. If densities and the surviving sex ratio reflect intrinsic local adaptation, one would expect succeeding annual sex ratios observed on individual trees to be autocorrelated. Although eight of ten trends were positive, only one of ten between-



Tree	A	B	C	D	Tree
183	.225	> .054	.091	< .112	184
185	.040	> .030	.035	< .108	186
187	.171	> .117	.129	< .190	188
189	.073	< .116	.092	< .113	190
199	.123	> .054	.097	< .118	200
26	.135	> .048	.086	> .074	27
29	.161	> .076	.113	< .146	38
30	.169	> .076	.077	< .126	31
32	.106	< .127	.056	< .112	33
35	.094	> .058	.058	< .069	34
37	.196	> .153	.052	< .068	36

Figure 2. Late-instar sex ratios from near and far sides of adjacent tree pairs. Each row represents four samples from a single pair of pines. Columns A and D show sex ratios from the far sides, while B and C are from the near sides. Cases violating the prediction are highlighted. Redrawn after Alstad & Edmunds 1983b. © Academic Press, Inc.

year comparisons of successive sex ratios on individual trees showed a significant rank correlation (1979-1981, $n = 11$, $r_s = 0.683$, $p < 0.05$), and one more approached significance (1979-1980, $n = 11$, $r_s = 0.524$, $0.05 < p < 0.10$) (Alstad and Edmunds, 1989). Year-to-year reversals in the rank order of sex ratios and densities on individual trees in Figure 3 illustrate this point. The weakness of year-to-year density correlations suggests that selection pressures change seasonally. Tree genotypes remain constant over time, so the selection process driving these patterns of insect survival is likely to be an extrinsic, rather than an intrinsic attribute of the host tree. These data also suggest that extrinsic factors are “noisy,” causing substantial mortality (and selection), but varying over such short time periods that adaptive responses may correspond only to long-term averages.

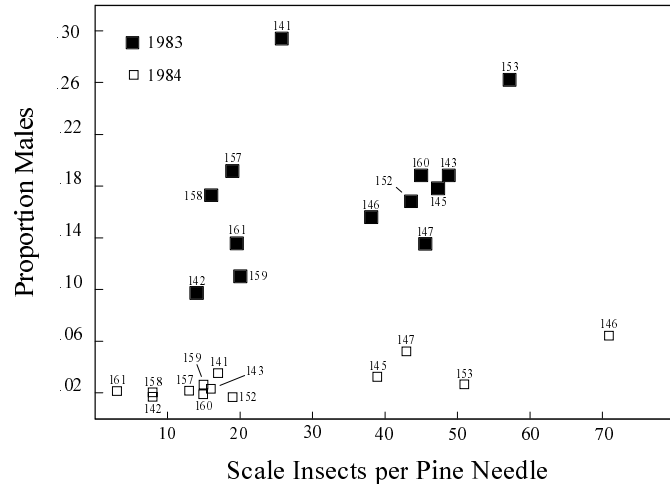


Figure 3. Late-instar sex ratios with scale density during the 1983 and 1984 seasons. Numbers identify individual trees for comparison. Redrawn after Alstad & Edmunds 1989.

Long-term observations also provide anecdotal information about the importance of weather in the interaction between scales and pine. There has been a damaging population density of scale insects adjacent to apple and pear orchards at Dryden since the middle 1950's. When I began work there in 1985, many trees were heavily infested and almost all of the pines carried some scales. The summer of 1989 was drier than usual (Figure 4), and precipitation that fall and winter (when most of the annual water budget accumulates) was about 25% of its 10-year average. In the summer of 1990 scale insect densities increased dramatically, and by 1991 all of the trees at that study site were dead. A similar anecdote is available from our field site at The Dalles. When I first began work there in 1979, I feared that I would soon have to abandon the site because scales would overwhelm and kill all the pines. Seven years later in 1986, scale densities had fallen to the point where I had difficulty finding sufficiently infested trees for my experiments, and almost all of the pines were in much better condition. In the ensuing decade scale densities have recovered. The nearest weather records are much farther from this location, so I cannot cite rainfall data like those in Figure 4, but both of these examples demonstrate that extrinsic factors have a strong influence on the fitness of scale

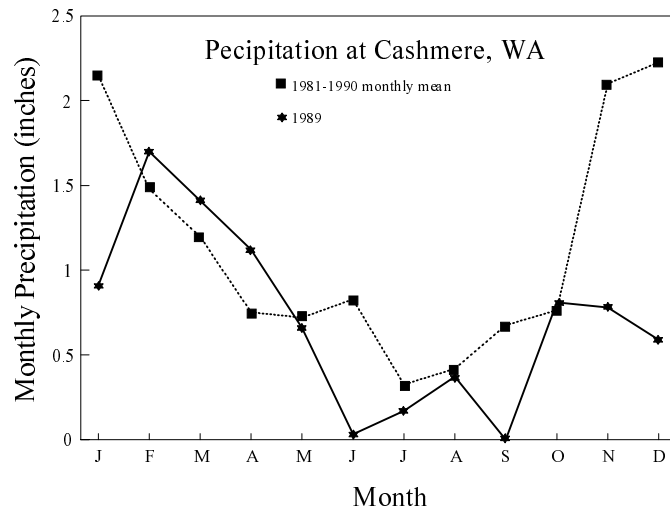


Figure 4. Precipitation at a gauging station 10 km from the study site at Dryden, WA where a 1989 drought led to increased scale insect density and widespread pine mortality. Data provided by Ellis Darley.

insects and their interaction with pines.

Allozyme Studies of Population Genetic Structure

The drift hypothesis suggests that scale insect populations are sufficiently viscous (i.e., the insects move so little) that local differentiation can develop by chance. To test this, we chose thirteen infested pines from three plots on adjacent city blocks at the Spokane study site, and collected five twig samples from each tree for a hierarchical analysis that partitioned the insects' genetic variance among plots, among trees within plots, and among twigs within individual trees within plots. All 13 trees grew within a radius of 100 meters. After investigating 30 enzyme systems, we found three with sufficient genetic variation to be useful, acid phosphatase (*Acp*, EC 3.1.3.2), 6-phospho-glucose isomerase (*Pgi*, EC 5.3.1.9), and NADP-dependent malate dehydrogenase or "malic enzyme" (*Me-1*, EC 1.1.1.40). Because the average persistence of neutral alleles is proportional to effective population size, it is not surprising that periodic founder effects, haplodiploidy, and extreme polygyny would reduce the genetic variance of black pineleaf scale insects relative to most diploid, sexual species (Crow & Kimura 1970).

We used allozyme data from 2,350 diploid female scale insects to estimate two of Wright's F statistics. The fixation index, F_{ST} (Wright 1951, 1965, 1978; Weir & Cockerham 1984; Weir 1990), gives a standardized genetic variance between subpopulations, normalized against the limit to that variance at the observed allelic frequencies.

$$F_{ST} = \frac{\sigma^2}{\bar{p}\bar{q}}$$

It can also be interpreted as a measure of the heterozygote deficiency associated with subdivision of a population into drifting, genetically isolated demes,

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

where H_T is the expected heterozygosity for the entire population, calculated as $2\bar{p}\bar{q}$ using global allelic frequency estimates, and H_S is the expected heterozygosity of demic subunits calculated as a weighted average that incorporates corrections for subunit size and allelic frequency (Nei 1977, 1978). In either case, F_{ST} varies inversely with interdemic gene flow, taking values from 0 to 1. At both *Acp* and *Pgi* loci the F_{ST} calculations show significant genetic differentiation between insects sampled from the three pine plots and from different trees within plots (Table 3). Wright (1951) showed that the number of migrant exchanges (N_{em}) is inversely proportional to F_{ST} , so these allozyme data from black pineleaf scale indicate about 2-15 between-tree migrant exchanges per generation. In 5 of 13 cases F_{ST} estimates at the *Pgi* locus also show significant differentiation between twigs sampled only a few meters apart on the same tree (Alstad & Corbin 1990).

F_{IS} estimates the deviation of observed heterozygote numbers (H_I) from those expected (H_S) on the basis of Hardy-Weinberg equilibria (Wright 1965, 1978;

$$F_{IS} = \frac{H_S - H_I}{H_S}$$

Table 3. Hierarchical F_{ST} values among plots, among trees within plots, and among twigs within trees within plots. Sample-size limitations permitted only a two-level analysis for *Me-1*. Significance of deviations from 0 was calculated as $X^2=2NF_{ST}$, where N is the number of individuals, and df is 1 less than the number of subpopulations sampled (Neel & Ward 1972). Data from Alstad & Corbin 1990.

	<i>Acp</i>	<i>Pgi</i>	<i>Me-1</i>
F_{ST} among plots	0.0233***	0.1123***	0.0527***
F_{ST} among trees			
within Plot I	0.0289***	0.0874***	0.0830*
within Plot II	0.0241**	0.0741***	0.0047ns
within Plot III	0.0106*	0.1216***	0.0010 ns
F_{ST} among twigs			
within Plot I			
within Tree 142	0.0000 ns	0.0000 ns	
within Tree 143	0.0000 ns	0.0000 ns	
within Tree 145	0.0033 ns	0.0647 ns	
within Tree 146	0.0170 ns	0.0652 ns	
within Tree 147	0.0305 ns	0.0086 ns	
within Tree 191	0.0418 ns	0.1311*	
within Plot II			
within Tree 152	0.0241 ns	0.0474 ns	
within Tree 153	0.0235 ns	0.1022**	
within Plot III			
within Tree 157	0.0023 ns	0.1207**	
within Tree 158	0.0298 ns	0.0149 ns	
within Tree 159	0.0032 ns	0.1269***	
within Tree 160	0.0019 ns	0.0000 ns	
within Tree 161	0.0000 ns	0.2826***	

with expectations adjusted for sample-size and frequency biases according to the method of Nei, 1977, 1978). Positive F_{IS} values indicate a heterozygote deficiency relative to the Hardy-Weinberg expectation, and negative values an excess. In a hierarchical analysis of population genetic structure, F_{IS} is conceptually equivalent to an F_{ST} estimate made one step lower in the spatial hierarchy. Procedurally however, F_{IS} has more statistical power because it compares the entire data set with a formal null model (binomial expectation), while F_{ST} evaluates the variance among subsets. Genotypic distributions at the tree level gave F_{IS} values for *Acp* and *Pgi* that were significantly positive in 9 and 11 of the 13 cases, respectively, demonstrating heterozygote deficiencies, nonrandom mating, and pervasive substructure within host trees. F_{IS} estimates from individual twigs showed little statistically significant deviation from genotypic equilibrium, suggesting that random-mating, demic units typically encompassed twigs or branches within individual host trees (Alstad & Corbin 1990).

To appreciate the extraordinary structure that these data imply, it is interesting to compare them with the 1987 analysis by McCauley and Eanes of the sedentary and geographically differentiated

milkweed beetle *Tetraopes tetraophthalmus*. Standardized genetic variances and statistical confidences for the two data sets are comparable, but the regions of McCauley and Eanes comprised the states of Virginia, Tennessee, Illinois, New Hampshire and New York, while ours were portions of a single hectare on the northern edge of Spokane. Black pineleaf scale insects show demic differentiation over extremely short distances.

What about the malic enzyme locus? An analysis of population structure using Wright's F -statistics requires that the genetic markers be selectively neutral. To test this assumption, we compared heterozygosities and allelic frequencies for each enzyme system with two components of insect fitness, local scale density and the surviving sex ratio. Neither allelic nor genotypic frequencies at the *Acp* and *Pgi* loci were related to these indices of scale performance, and we have no evidence that selection affected the pattern of their variation. In contrast, the density achieved by scales on different trees correlated with frequencies of the most common *Me* allele (Figure 5, $n = 13$, $r = 0.652$, $p = 0.016$). There was also a significant association between the sex ratio at mating and the frequency of this same allele ($n = 13$, $r = 0.583$, $p = 0.037$) (Alstad & Corbin, 1990).

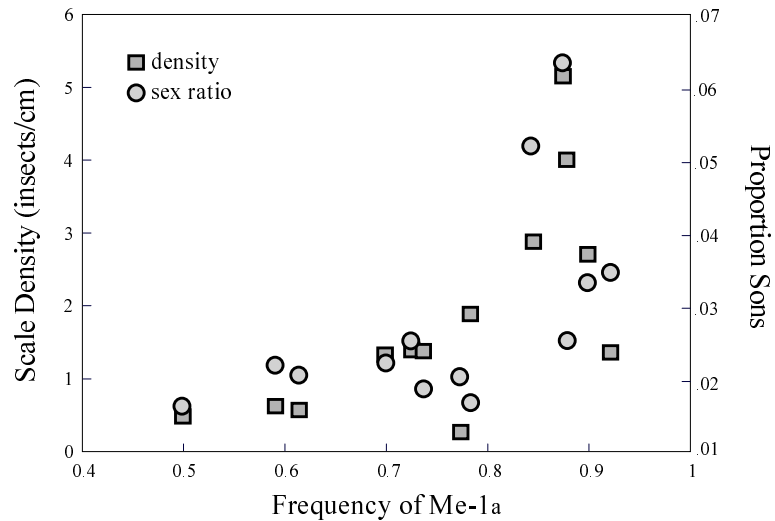


Figure 3. Density and late-instar sex ratio of surviving scale insects collected in early May from 13 pines, plotted against frequencies of the most common malic enzyme allele. Density and sex ratio estimates were made by counting insects on 30 needles per twig from 5 twigs sampled for the allozyme study. Redrawn after Alstad & Corbin 1990.

These correlations suggest that we have identified either a locus under selection, or (more likely) a hitch-hiker linked in disequilibrium with some other selected locus. They compromise the malic enzyme system as an indicator of population structure because selection might affect genotypic frequencies within our samples; for example, allelic frequency differences between the sexes resulting from the asymmetrical survival of males and females might inflate heterozygosities, producing Hardy-Weinberg equilibria in the F_{IS} analyses despite pervasive substructuring, and causing F_{ST} values for this locus to be overly conservative estimates of local differentiation (Crozier 1985, Hartl and Clark 1989). At the same time, the apparent selection on *Me* suggests a new hypothetical mechanism of potential local adaptation. Scale densities were low on trees with the highest *Me* variance, and increased with Me^a frequencies ranging from 0.6 to 0.9, suggesting a Density-Dependent Selection Hypothesis. Figure 5 suggests that Me^a has a competitive advantage over Me^b on chlorotic needles that have been heavily damaged by scale feeding. In effect, scale insects themselves may be an important extrinsic factor, altering the foliage and the selection pressures consequently imposed on the insect herbivores.

Discussion

The first conclusion to be drawn from these collected data and field observations is that the neutral Drift Hypothesis is alive and well in Black Pineleaf Scale insects. Two polymorphic marker loci demonstrate barriers to random mating between trees, and even between the twigs of individual trees. Black Pineleaf Scale populations are genetically differentiated over very short distances, and the interdemic variances suggest that only a dozen or so migrant exchanges occur between trees in each generation. Counter hypotheses based on some selection process would have to explain concordant allelic frequency variation and heterozygote deficiencies in two different marker systems while the individual twig samples stay in genotypic equilibrium; they offer a less parsimonious explanation for our data. This result is fully consistent with the viscous mating system and natural history of scale insects, which afford considerable scope for neutral evolution. Since many of the taxa in which ecologists have inferred local adaptation have sedentary habits (Mopper 1996), this may be a fairly general result.

The Intrinsic Local Adaptation Hypothesis, that insects evolve in response to genotypes of individual host trees, has become progressively less tenable as we have learned more about the pine/scale system. It is directly falsified by the reciprocal transfer results of Tables 1 and 2, which show receptor main effects but neither donor main effects nor interactions. One hypothetical explanation for this result emerges from our long-term analyses of scale population biology. Changes in density and the scale insect sex ratio within the course of a single generation demonstrate powerful selective forces; there is very heavy mortality associated nonrandomly with ploidy, and hence insect genotype. The problem with respect to Intrinsic Local Adaptation is that these strong selective forces vary independently of plant genotype. When we first began to keep records on marked trees, we saw three consecutive years in which insect density and the surviving sex ratio increased annually on every tree, and interpreted these measures as indices of increasing local adaptation (Alstad & Edmunds 1983a); then in the fourth year both densities and sex ratios crashed precipitously (Alstad & Edmunds 1989). The fact that late-instar sex ratios on individual trees showed little significant correlation from year to year over a long time series suggests that unpredictable environmental changes contribute variance that may limit the scales' selection response to the constant, intrinsic characteristics of their host.

The transfer experiment reported by Edmunds & Alstad (1978) showed evidence of local adaptation while the reciprocal experiment summarized in Tables 1 and 2 did not. There were extrinsic environmental variations (as well as genotypic differences) between donor and receptor trees in the 1978 experiment, but only intrinsic genotypic treatments in the later, reciprocal design. This implies that extrinsic differences between the trees at Spokane and Deer Park contributed to variation in insect survival. Allozyme data support the Extrinsic Local Adaptation Hypothesis more directly, suggesting that scales themselves can alter the selection regime. Scale feeding causes chlorotic lesions, and at moderate scale density these lesions merge into a continuous band affecting up to 80% of each pine needle. Under these conditions, elongation of successive annual needle crops is stunted and tree condition deteriorates (Edmunds 1973). It is not surprising that this damage might produce density-dependent changes in selection pressure. In this case the *Me^a* allele is at high frequency on chlorotic pines and at lower frequency on green ones, with a tree-to-tree frequency range of 40%. We do not know whether the pattern persists from generation to generation (a requirement of local adaptation, Jaenike 1981), but it seems

unlikely that natural selection would cause such extreme changes in the course of a single season. These data thus suggest extrinsic local adaptation mediated by the effect of insect feeding on the tree phenotype.

In conclusion, after 20 years' field research with black pineleaf scale and ponderosa pine, the neutral Drift Hypothesis and the Extrinsic Local Adaptation Hypothesis remain viable. The Intrinsic Local Adaptation Hypothesis is dead. Tree genotypes do not function in isolation, and it might seem that I have killed a straw man by defining intrinsic host traits so narrowly as to exclude attributes affected by genotype * environment interaction. There are both conceptual and practical reasons for the narrowly drawn hypothesis. It is the genotype *per se* that is interesting to biologists with respect to parasites, recombination and sex; and it is genotype *per se* that is a plant adaptation, defining the limit to broad-sense heritability of traits that influence insect performance. Finally, it is host genotype that gives us a theoretical basis for experimental designs. Transfer experiments are only instructive if we understand the pattern of selection pressures that drive local adaptation; tree genotype sets an *a priori* expectation for these spatial limits. If the genotypes of individual trees do not define the spatial distribution of the pertinent selection processes, then tree-to-tree transfer experiments yield inconsistent results. Tree-to-tree variation in insect density originally led us to an interest in local adaptation; but insect density also varies within trees (between shaded interior and sunlit peripheral branches). In retrospect, parasites, recombination and sex lured us initially to the wrong spatial dimension, and it took awhile to straighten things out.

Can we scale up a pine to view the world? Plants exert very powerful selection pressures on insect herbivores, these pressures can be locally variable, and the panmictic neighborhood sizes of many sedentary insects are small. I continue to believe that the natural history of plant/insect interaction holds many examples of local adaptation. Experience with black pineleaf scale on ponderosa pine has shown that the spatial pattern of environmental variation sufficient to affect insect herbivores is also small, and that much local adaptation will thus be mediated by extrinsic causes. This is unfortunate for the discipline, because (a) the extrinsic hypothesis has fewer coevolutionary consequences, (b) the mechanistic diversity of extrinsic effects will confuse the theoretical bases of productive research and render the results a catalog of special cases, and (c) extrinsic variation (along with neutral evolution) will increase the noise-to-signal ratio of intrinsic local adaptation mediated by plant genotype.

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