SPATIAL DISTRIBUTION OF DEFENSE CHEMICALS AND MARKERS AND THE MAINTENANCE OF CHEMICAL VARIATION

Rose L. Andrew,[1] Rod Peakall, Ian R. Wallis, and William J. Foley

School of Botany and Zoology, The Australian National University, Canberra, ACT 0200 Australia

Abstract. Exploring the spatial distribution of variation in plant secondary metabolites is critical for understanding the evolutionary ecology of biochemical diversity in wild organisms. In the present study, concentrations of foliar sideroxylonal, an important and highly heritable defense chemical of *Eucalyptus melliodora*, displayed strong, fine-scale spatial autocorrelation. The spatial patterns observed could promote associational effects on herbivore foraging decisions, which may influence the selection pressures exerted on sideroxylonal content.

Multiple chemical traits have roles in certain eucalypt–herbivore interactions, and the spatial characteristics of the herbivore foraging environment are therefore determined by these different factors. We used a model of *E. melliodora* intake by common brushtail possums (*Trichosurus vulpecula*), based on the combined effects of two chemical traits, to explore this idea and found that the spatial patterns were different to those of sideroxylonal alone.

Spatial genetic autocorrelation, examined using microsatellites, was strong and occurred at a fine scale, implying that restricted gene flow might allow genetic patches to respond to selection relatively independently. Local two-dimensional genetic autocorrelation, explored using a new heuristic method, was highly congruent with the pattern of local phenotypic variation observed for sideroxylonal, suggesting that the genetic variance underlying the sideroxylonal variation is similarly structured.

Our results suggest that the spatial distribution of genetic and phenotypic variation could influence both the selective pressure imposed by herbivores on eucalypt defenses and the potential of populations to respond to natural selection. Spatial context should be considered in future studies of plant–herbivore interactions.

Key words: common brushtail possum; Eucalyptus melliodora; *genetic variation; herbivory; 1,8-cineole; phenotypic variation; polyethylene glycol (PEG)-binding capacity; sideroxylonal; spatial autocorrelation; spatial heterogeneity;* Trichosurus vulpecula.

INTRODUCTION

Phenotypic variation is extremely important in the ecology and evolution of plants, as it affects both intra- and interspecific interactions. Competition, pollination, and herbivory are among many ecological processes influencing the fitness of a plant, and all of these may vary with plant phenotypes (Cariveau et al. 2004, Cahill et al. 2005, Moore and Foley 2005). Spatial factors can be critical to each of these processes, and although there is strong evidence of the importance of the spatial distributions of different plant species (e.g., Baraza et al. 2006), complex spatial patterns also occur in intraspecific variation and may have a similar influence (Klinkhamer et al. 2001). The factors producing spatial structure in plant phenotypes should thus be considered as moderators of the ecological and evolutionary processes involving plants. Restricted dispersal and unequal propagule production, in addition to environmental effects on phenotypes, can cause spatial structure

in phenotypic variation, thereby affecting competition, pollination, and herbivory.

Strong intraspecific variation in defense chemical concentration or composition occurs in many plant species and is often heritable (Carroll et al. 2000, Orians et al. 2003). There has been marked progress in our understanding of the effects of secondary chemicals on herbivores, organisms at higher trophic levels, and pollinators, and of the selection pressures acting on plant defenses (Agrawal et al. 2002, Strauss et al. 2004, Moore and Foley 2005). The benefits that plants obtain by producing chemical defenses depend on the propensity of herbivores to feed less on more strongly defended plants. Herbivores must select foodstuffs to meet nutritional requirements, while minimizing the ingestion of potentially toxic compounds. However, the decision-making process is complex and based on many factors, including food quality, predator avoidance, social and reproductive requirements, and the proximity of alternative food sources (Searle et al. 2005). The importance of spatial context in herbivore foraging behavior has long been recognized as a component of the costs in optimal foraging models (Pyke 1984). The spatial arrangement of plants with different levels of defense can alter herbivore behavior (Hjältén et al. 1993, Alm

Bergvall and Leimar 2005, Bergman et al. 2005, Alm Bergvall et al. 2006), as the amount of feeding on any given plant depends on the perceived cost or benefit of moving to a new food source (Searle et al. 2005). Such behavior is important from the perspective of plant defense evolution because it is expected to influence selection pressure on defenses. The scope for such behavior to influence plant secondary metabolite evolution in nature is largely unknown, however, as there are very few studies of the spatial structure of defense phenotype variation in the wild (Covelo and Gallardo 2004, Brenes-Arguedas and Coley 2005). Spatial autocorrelation analysis is an effective method for exploring the intensity and extent of spatial structure in the phenotypic variation within a population (Legendre and Fortin 1989).

Genetic variation is essential for populations and species to evolve in response to natural selection, and many plants display significant genetic variation in herbivore defense traits (Geber and Griffen 2003). In the case of heritable traits, spatially structured phenotypic variation can be due to the underlying spatial genetic structure, which itself is determined by patterns of gene flow and recruitment. Restricted dispersal is common in plants and results in spatial genetic autocorrelation, with limited gene flow among spatially clustered groups of individuals in continuous populations. This may provide the genetic isolation required to respond independently to natural selection. The use of neutral genetic markers to study spatial genetic structure within populations is useful because it can not only explain the spatial distribution of phenotypic variation, but also identify the scale of genetic patchiness.

Foliar sideroxylonal and 1,8-cineole are important antifeedants for a range of mammalian and insect herbivores of *Eucalyptus* L'Hér., but they are highly variable in concentration within populations (Lawler et al. 2000, Wallis et al. 2002, Moore et al. 2004, Andrew et al. 2005). We have previously shown that the concentrations of sideroxylonal, cineole, and foliar nitrogen are strongly heritable in the wild (Andrew et al. 2005). As natural selection is expected to reduce heritability over time (Mousseau and Roff 1987), the high heritability of sideroxylonal and cineole is at odds with their apparent importance in deterring herbivores. This may suggest that natural selection on these traits is moderated by factors such as temporal and spatial heterogeneity in herbivory. Alternatively, the spatial distribution of phenotypic and genetic variation could also influence both the selection pressure exerted by herbivores and the response of the population to this selection.

Here we explore the local spatial patterns of genetic and phenotypic variation in yellow box, *Eucalyptus melliodora* A. Cunn. ex Schauer, using spatial autocorrelation analysis and related methods. We ask several questions:

1) Is variation among trees in the concentration of sideroxylonal spatially structured?

2) Is spatial structure in sideroxylonal concentration constant across the population?

3) Are spatial patterns congruent across defense traits? We consider additional chemical traits important to some herbivores. These are foliar 1,8-cineole concentration, polyethylene glycol (PEG)-binding capacity of the foliage (a functional measure of the effectiveness of foliar tannins in deterring herbivores), and total foliar nitrogen concentration.

4) Is spatial structure different when multiple traits are considered? As an example of how spatially structured variation in multiple chemical traits might affect a herbivore, we used captive feeding data to predict how much a brushtail possum (*Trichosurus vulpecula*) would eat from each tree in the natural *E. melliodora* population.

5) Do neutral microsatellite markers display fine-scale spatial genetic structure, congruent with phenotypic variation?

In our discussion of the results, we ask whether the distributions of defense chemicals could contribute to the maintenance of biochemical diversity in this species by promoting associational resistance. Finally, we ask if the response of defense chemicals to the selective pressures exerted by herbivores could be limited by the variable spatial structure of genetic variation.

METHODS

Sample collection and analysis

The study site and sample collection procedure were described in Andrew et al. (2005). *Eucalyptus melliodora* is a common woodland and pasture tree in southeastern Australia, but the study population at Mulligan's Flat (Australian Capital Territory), which is part of Canberra Nature Park, is one of the largest intact patches of yellow box/red gum grassy woodland. In a 6-ha area, all trees with a trunk circumference at 1.3 m of at least 27 cm were sampled. This was an arbitrary cutoff, corresponding roughly to the size of the smallest flowering trees.

Microsatellite amplification for six loci and the measurement of foliar chemical characters using near-infrared spectroscopy (NIRS) were described in Andrew et al. (2005). Here we focus on the concentration of sideroxylonal, because it has been shown to be important in both mammalian and insect herbivory, but 1,8-cineole (hereafter called cineole) concentration, PEG-binding capacity, and total nitrogen concentration were also analyzed. Cineole concentration is positively correlated, both phenotypically and genetically (Andrew et al. 2005), with sideroxylonal and this helps herbivores to avoid sideroxylonal-rich leaves (Lawler et al. 1999).

In total, 255 trees were sampled; however, full genotypes were obtained for only 232 trees. For consistency between analyses of phenotypic and genotypic data, only these trees were included in the analyses that follow. To investigate the effect of tree size on foliar chemical traits and spatial structure, the population was

split into two size classes of 116 trees based on percentiles of dbh (diameter at 1.3 m). Most analyses were conducted on these data sets separately, in addition to analyses of the whole population. Size class 1 contained trees with dbh of 8.6–16.9 cm; size class 2 contained trees with dbh of 17.2–180 cm.

Global spatial autocorrelation analysis of phenotypes

Spatial autocorrelation analysis was used to identify spatial structure by testing the relationship between similarity and spatial proximity, following the method of Smouse and Peakall (1999). This method can be applied to univariate phenotypic data and multivariate genetic data, as long as an appropriate squared distance matrix is used as the input.

In the present study, a squared Euclidean phenotypic distance matrix was computed comparing all possible pairs of individuals in the population for each trait. Global spatial autocorrelation analysis was performed on these distance matrices using 20-m distance classes in GenAlEx 6 (Peakall and Smouse 2006). An autocorrelation coefficient, r, was computed for all pairs of individuals falling within each distance class and was tested against a null hypothesis of no spatial structure by permutation. In order to statistically compare the fine-scale spatial autocorrelation across phenotypes and tree size classes, we used bootstrapping to estimate 95% confidence intervals around autocorrelation coefficients for the first distance class, with cutoffs ranging from 10 to 50 m. Increasing the upper limit of the distance class improved statistical power by increasing the number of pairwise comparisons included.

Mapping of phenotypes and local variation in phenotypes

We examined the distribution of trees with high and low concentration of sideroxylonal, cineole, PEG-binding capacity, and nitrogen by plotting their positions on a map of the population. Various approaches exist for representing the spatial distribution of variation, including several methods of smoothing. In the case of E. melliodora, however, smoothed phenotypes are unsatisfactory due to the patchy distribution of trees. Instead, we highlighted the most and least defended trees.

To determine whether the spatial structure was constant across the population, we calculated the local standard deviation for each tree and its 10 nearest neighbors using a Fortran 95 program written for this purpose. The trees with the highest and lowest 25% of local standard deviations were highlighted on the map of the population. Although local autocorrelation can also be applied to univariate phenotypic data, the centers of autocorrelation identified are more likely to be patches of extreme values, as autocorrelation is lower when neighboring trees with similar phenotypes are close to the population mean. Because we are interested in how variable a patch might appear to herbivores, the local

standard deviation was a more appropriate indication of variability.

Global spatial autocorrelation analysis of microsatellite data

Global spatial autocorrelation analysis with GenAlEx 6 (Peakall and Smouse 2006) was performed on the multivariate microsatellite data to study the spatial genetic structure of the entire study population. Permutation and bootstraps were used for testing of autocorrelation coefficients and estimation of 95% confidence intervals, respectively (Smouse and Peakall 1999, Peakall et al. 2003).

Local spatial autocorrelation analysis of microsatellite data

Global autocorrelation analysis summarizes the spatial structure of the entire study population, but does not indicate the relative contributions of individuals or groups of individuals to the overall pattern. Positive global autocorrelation between nearby individuals may result from more-or-less uniform decay of genetic similarity with increasing distance, or from some individuals exerting a particularly strong influence on overall structure. Local spatial autocorrelation uses subsets of the available data to investigate spatial autocorrelation in different parts of the population (Anselin 1995). Recently, Double et al. (2005) presented a heuristic method for exploring local spatial autocorrelation in multivariate genetic marker data and used it to identify patterns consistent with strong reproductive skew and sex-biased dispersal in the Superb Fairy-wren, Malurus cyaneus. This approach, which is a modification of the method of Smouse and Peakall (1999), takes each individual and calculates local spatial autocorrelation, r_{loc}: the autocorrelation coefficient of the comparisons involving this focal individual and its n nearest neighbors.

Two-dimensional local spatial autocorrelation analysis (2D LSA) was performed in GenAlEx 6 (Double et al. 2005, Peakall and Smouse 2006). Each r_{loc} estimate was compared with a null distribution with no spatial structure, constructed using 10 000 permutations. Trees were compared with 1–30 nearest neighbors to measure local spatial autocorrelation (r_{loc}) and to determine the consistency of the observed pattern.

Integrating chemical traits to predict feeding by common brushtail possums

In order to link the chemical traits that we measured for each tree to their potential effect on the feeding preferences of a herbivore, we reanalyzed part of the data presented by Wallis et al. (2002) to provide predictions of palatability for each tree in the population. The earlier study related the chemical composition of E. melliodora foliage to the feeding rates of common brushtail possums (Trichosurus vulpecula) in captivity. Common brushtail possums are generalists whose diet

can contain understory species, but they favor eucalypts of subgenus *Symphyomyrtus* (including *E. melliodora*), which comprise ~60% of their diet (Kerle 1984; J. DeGabriel, *personal communication*). In the study of Wallis et al. (2002), six to eight brushtail possums were fed 26 trees in two experiments based on Latin-square designs.

Sideroxylonal, cineole, and total nitrogen concentrations were measured at the time of the study and concentrations of sideroxylonal and cineole were strongly collinear (Wallis et al. 2002). Because the PEG-binding capacity of leaves is also an important determinant of palatability for these animals (Marsh et al. 2003), we applied an NIRS model to predict this trait from scans of leaf material taken at the time of the feeding trial. PEG inactivates foliar tannins by binding to the functional groups that would otherwise bind protein in the diet and reduce nitrogen digestion.

Based on these chemical data, we developed a mixed model with REML (residual maximum likelihood) estimation in GenStat Version 8 (GenStat 2005) to predict the potential feeding rates of common brushtail possums (measured as dry matter intake, DMI), on each tree in our study population. The full model contained "experiment," "sideroxylonal," "nitrogen," and "PEG-binding capacity" as fixed effects and "experiment $\times$ possum" and "experiment $\times$ day" as random variables. Interactions among the main fixed effects were considered initially and terms were dropped sequentially if they were not significant according to Wald tests.

The heritability of DMI predictions was estimated using the regression method of Ritland (1996), with spatial distance and relatedness as predictors and using permutation testing as described in Andrew et al. (2005). Relatedness was estimated using the symmetric version of the Lynch and Ritland (1999) estimator and separate analyses were performed with and without a 60-m distance cutoff (Andrew et al. 2005). Spatial analysis was conducted on predicted DMI as for the chemical traits.

RESULTS

Correlation of leaf chemistry with tree size

Foliar concentrations of sideroxylonal and cineole had high coefficients of variation, whereas those for PEG-binding capacity and total nitrogen concentration were much lower (Table 1). Using GenStat Version 8 (GenStat 2005) to perform a two-sided test, the natural logarithm of dbh was uncorrelated with foliar nitrogen ($r = -0.05$, $P = 0.448$) and marginally correlated with PEG-binding capacity ($r = 0.120$, $P = 0.069$). Sideroxylonal was negatively correlated with ln(dbh) ($r = -0.264$, $P < 0.001$), whereas the correlation of ln(dbh) with cineole was weaker, but still significant ($r = -0.141$, $P = 0.032$).

Spatial analysis of phenotypes

Foliar concentrations of sideroxylonal, cineole, and the PEG-binding capacity of the leaves displayed

TABLE 1. Mean and coefficient of variation in 232 *Eucalyptus melliodora* trees for four chemical components (mg/g dry mass) and predicted daily dry matter intake (DMI, g dry matter/d) by common brushtail possums.

Variable	Mean	CV (%)
Sideroxylonal	17.9	29.6
Cineole	7.2	24.7
PEG-binding capacity	13.9	9.0
Nitrogen	13.6	6.8
Predicted DMI	30.1	19.9

significant spatial autocorrelation with a distance class size of 20 m, whereas foliar nitrogen concentrations did not (Fig. 1).

The separation of the confidence intervals changed as the size of the first distance class increased, but the overall pattern remained the same. The results of the analysis considering trees 0–40 m apart (i.e., combining the first two distance classes from the previous analysis), with estimation of 95% CIs estimated by bootstrapping, are shown in Fig. 2. The widths of the confidence intervals reflect the number of comparisons within the 0–40 m range: 4174 for all trees, 1390 for small trees only, and 792 for large trees only. Small trees displayed stronger spatial autocorrelation than large trees in all cases except nitrogen, and the difference was significant for sideroxylonal. In large trees, autocorrelation at this scale was only significantly greater than zero for total nitrogen concentration. When all trees were considered, sideroxylonal and cineole showed significantly stronger autocorrelation than did nitrogen, which was significant only when small trees were omitted. The foliar concentrations of cineole and sideroxylonal showed significant autocorrelation at this scale for both large and small trees, whereas the PEG-binding capacity of the foliage showed no significant spatial autocorrelation for large trees (Fig. 2). For small trees only, autocorrelation of PEG-binding capacity in this distance class was significantly lower than that of sideroxylonal.

The northern part of the population contains many of the low-sideroxylonal trees, although a cluster of trees with high sideroxylonal concentrations is nested within it (Fig. 3a). Cineole concentrations display a similar, but not identical, distribution (Fig. 3b). PEG-binding capacity is generally high in a large area to the south, but clustering of similar phenotypes is less apparent in the north than for sideroxylonal concentrations (Fig. 3c). Trees with high and low total nitrogen concentrations are intermixed through most of the population, with some weak clusters of similar phenotypes in the southeast and northwest of the population (Fig. 3d).

There were three areas of low variability in sideroxylonal concentration, based on 10 neighbors (Fig. 4a). One of these clusters was less apparent for cineole concentrations, which had low standard deviation in two additional areas (Fig. 4b). Areas of high standard deviation were similar for both sideroxylonal and
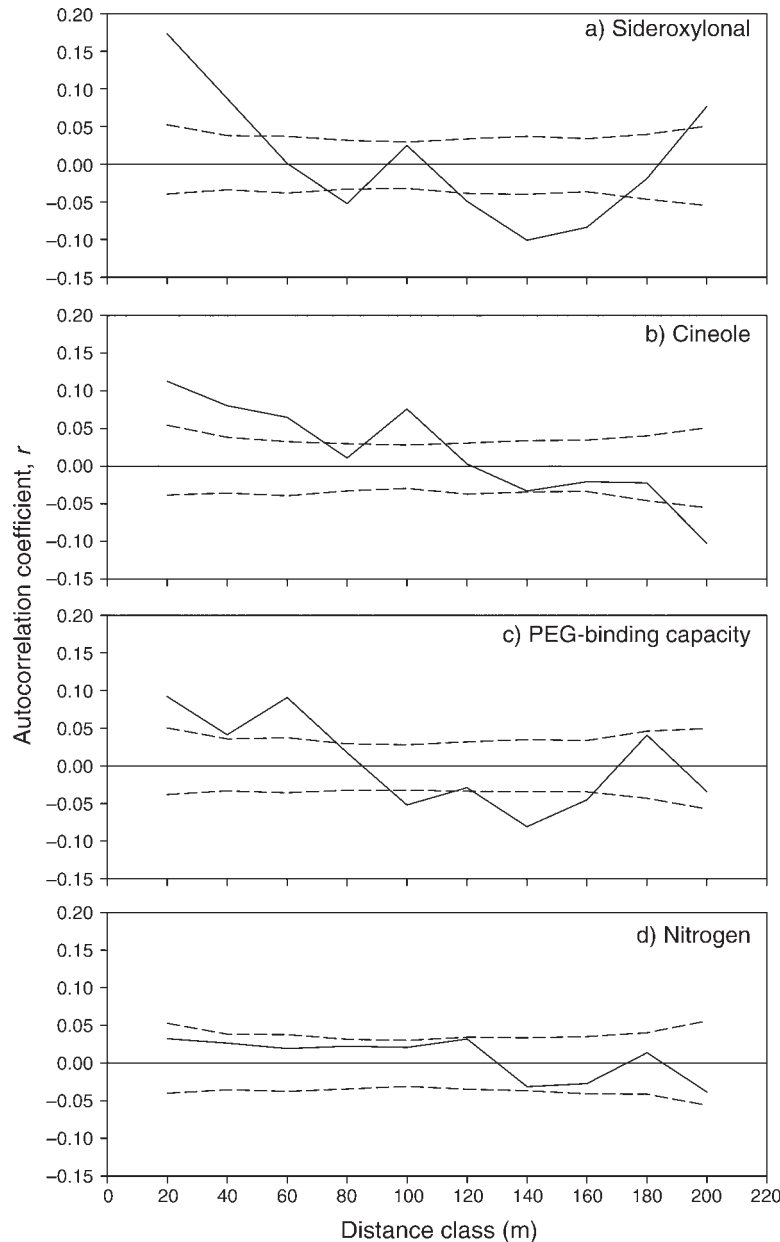
FIG. 1. Global spatial autocorrelation of phenotypic data for 232 *Eucalyptus melliodora* trees. Correlograms (solid lines) are shown with the boundaries of the 95% confidence interval (dashed lines) about the null hypothesis of no spatial structure, estimated by permutations. Distance classes of 20 m were used. PEG-binding capacity is a functional measure of the effectiveness of foliar tannins in deterring herbivores.

cineole. Variation in PEG-binding capacity was generally low in the southern half of the population, although two small clusters of low standard deviation occurred in areas which also had low sideroxylonal variability (Fig. 4c). Because spatial autocorrelation was low or nonsignificant for total foliar nitrogen concentrations (Fig. 2), the pattern of variability observed for this trait is probably due to the nonindependence of local standard deviation values for neighboring trees (Fig. 4d). The

lower (SD = 0.73) and upper quartiles (SD = 0.94) were both close to the population standard deviation (SD = 0.84).

Spatial genetic structure

Spatial autocorrelation analysis demonstrated significant global spatial genetic structure in the study population consistent with restricted pollen and seed dispersal, with positive autocorrelation between trees
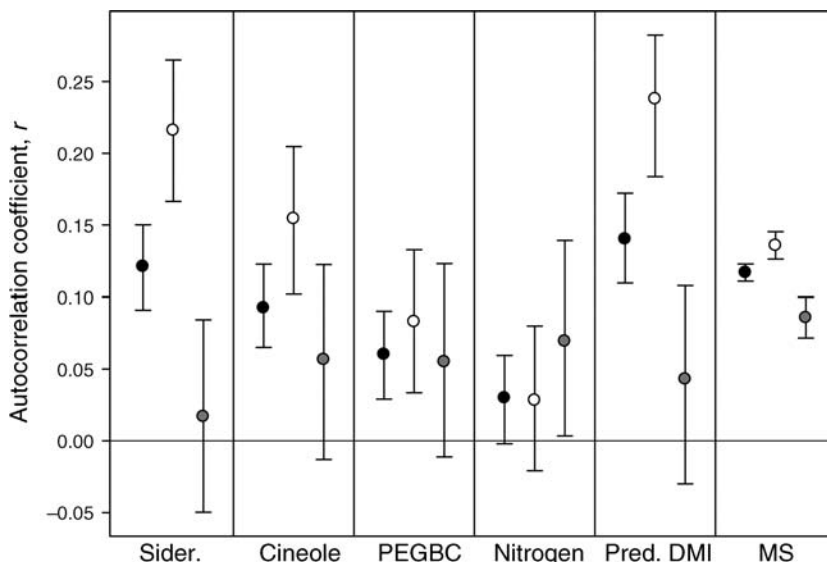
FIG. 2. A comparison of spatial structure in foliar chemical data, predicted dry matter intake by brushtail possums (Pred. DMI), and microsatellite data (MS). The chemical traits studied were sideroxylonal (Sider.), cineole and total nitrogen concentration, and the PEG-binding capacity (PEGBC) of leaves. Global autocorrelation coefficients were computed for the 0–40 m distance class, with 95% confidence intervals (indicated by error bars) estimated by bootstrapping. Separate analyses were conducted, including all trees (black circles), small trees only (white circles), and large trees only (gray circles).

within 60 m (Fig. 5). For the 0–40 m distance class, microsatellites displayed significantly stronger autocorrelation than did PEG-binding capacity and nitrogen concentration (Fig. 2). Autocorrelation was lower for microsatellites than for the concentration of sideroxylonal and predicted DMI when small trees only were considered. Although autocorrelation was significant for microsatellites in the large tree size class, it was not significantly different from that of any phenotypic trait (Fig. 2).

The mean distance to the nearest neighbor of a tree was 5.5 m, whereas mean distance to the 10th nearest neighbor was 18.8 m. Analysis by 2D LSA considering 10 nearest neighbors showed significant ($P < 0.01$) local autocorrelation for more than half of the trees in the population, using a one-tailed test. A more stringent cutoff of 0.000216 is required for an experiment-wide α of 0.05, under the assumption that the r_{loc} for trees are independent. However, this correction is unrealistically conservative, as r_{loc} values are not independent (Double et al. 2005). The map in Fig. 6 shows the positions of trees in the increasingly narrow tails of the permuted distribution, using six neighbors. There are two areas of high local autocorrelation in the north of the population, which are consistent when the number of neighbors considered vary, while the single significant trees in the southern part of the population depended on the number of neighbors considered.

Smaller trees showed a more pronounced pattern of local spatial autocorrelation than did larger trees. For six nearest neighbors, 48 small (41.4%) and 30 large (25.9%) trees had r_{loc} in the upper 1% tail of the permuted distributions. However, the northern autocorrelation hotspots were not merely produced by the small trees, as the positive r_{loc} values are concentrated in the north of the population for the large trees (not shown).

Predicted feeding by common brushtail possums

A summary of the model predicting the potential dry matter intake of *E. melliodora* foliage by common brushtail possums is given in Table 2. Nitrogen and the interactions between main terms were their effects were not significant. The expected dry matter intake (DMI, in grams of dry matter per day) by *T. vulpecula* of the trees in the study population under the conditions of the first experiment was calculated using the following formula, where sideroxylonal and PEG-binding capacity are both measured in milligrams per gram dry mass:

$$DMI = 89.47 - 0.84 \times sideroxylonal - 3.2 \times PEG - binding \ capacity.$$

The dry matter intake by brushtail possums predicted by this model was not significantly correlated with ln(dbh) ($r = 0.115$, $P = 0.080$). The heritability of predicted intake was marginally significant with no cutoff ($h^2 = 0.330$, $P = 0.058$), but not significant with a 60-m distance cutoff ($h^2 = 0.223$, $P = 0.122$). The effect of spatially structured environmental variation (b_e), estimated as the effect of increasing distance between individuals (in meters) on phenotypic similarity, was significantly negative in both cases (with no distance cutoff, $b_e = -0.004$, $P = 0.0004$; with a 60-m cutoff: $b_e = -0.002$, $P = 0.027$).
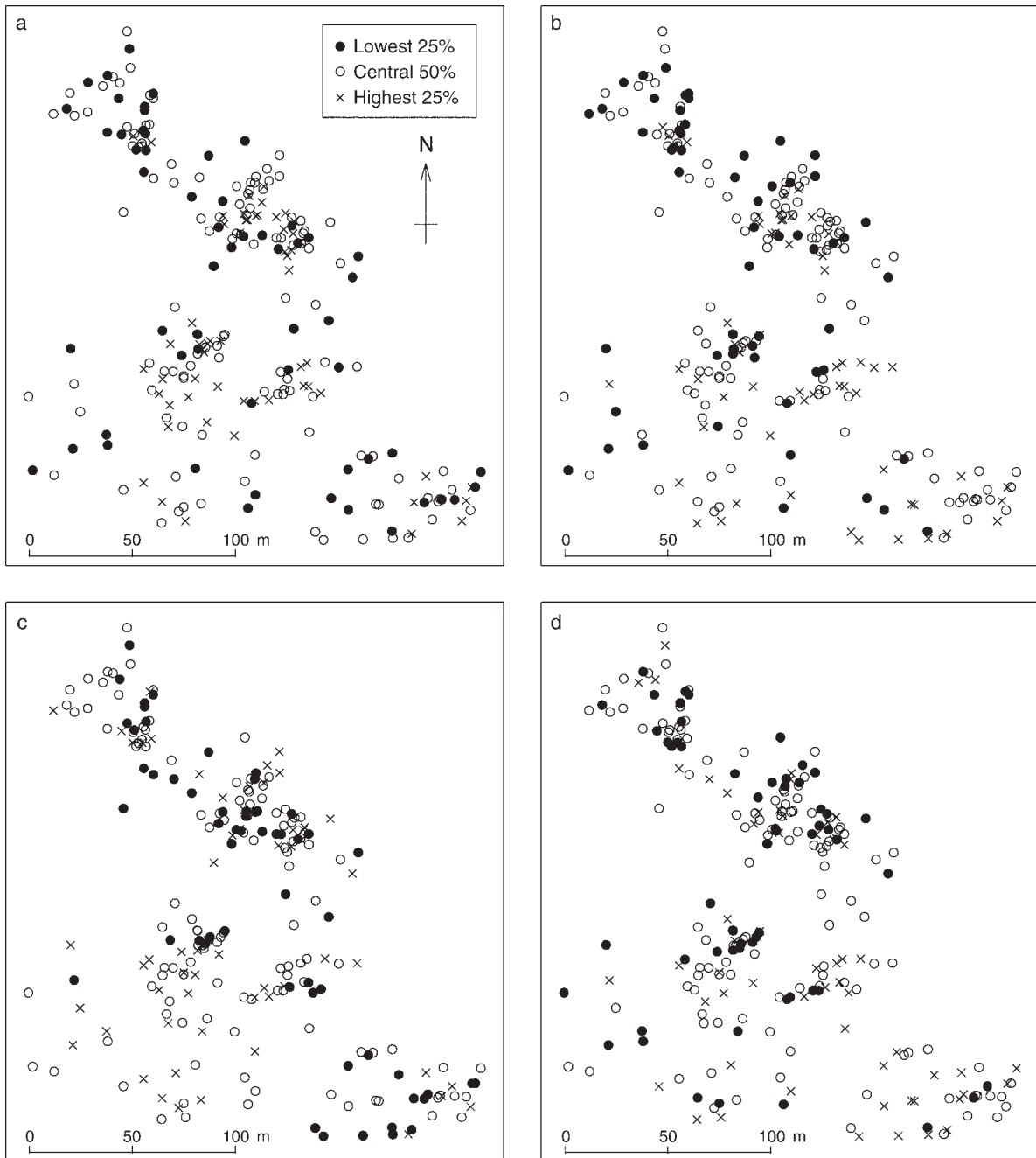
Fig. 3. Maps of the *E. melliodora* study population highlighting trees with high or low foliar concentrations of (a) sideroxylonal, (b) cineole, (c) PEG-binding capacity, and (d) nitrogen. On each map the lowest 25% of values are depicted as solid circles, values in the interquartile range (the 50% of values nearest to the median) are shown as open circles, and the highest 25% of values are represented by crosses. In panels (a–c), herbivores are expected to prefer trees with low concentrations, whereas the reverse is true for nitrogen (d). The population occupied a study area approximately 230 × 270 m.

Despite the larger coefficient for PEG-binding capacity in the predictive model, the distribution of variation in predicted potential DMI in the study population largely followed that of sideroxylonal (Fig. 7a), probably due to the greater variability of the latter in the study population (Table 1). However, certain clusters of trees

were more variable or less variable in predicted DMI than for either sideroxylonal concentration or PEG-binding capacity (Fig. 7b). Spatial autocorrelation analysis of predicted DMI also revealed stronger similarities with sideroxylonal concentration, although it was also significantly autocorrelated in the 40–60 m
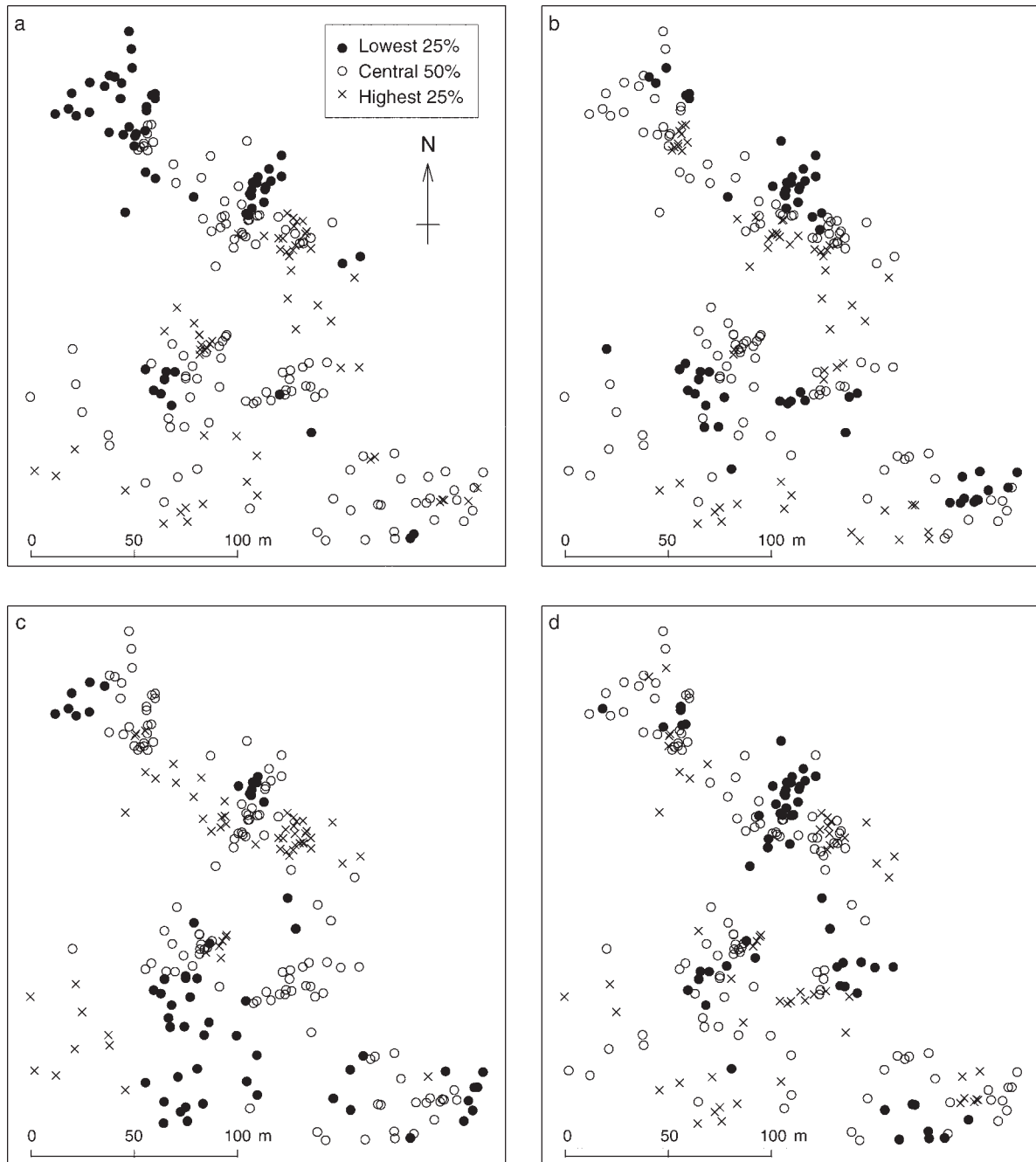
FIG. 4. Maps of the *E. melliodora* study population displaying trees with high or low local standard deviations of foliar chemical concentrations, calculated for the focal tree and its 10 nearest neighbors. Maps are shown for (a) sideroxylonal, (b) cineole, (c) PEG-binding capacity, and (d) nitrogen. On each map the lowest 25% of values are depicted as solid circles, values in the interquartile range (the 50% of values nearest to the median) are shown as open circles, and the highest 25% of values are represented by crosses.

distance class. With a distance class of 0–40 m, potential dry matter intake by possums showed significantly greater spatial autocorrelation than either PEG-binding capacity or total nitrogen concentration when the analysis was conducted on either all trees or small trees only (Fig. 7c).

DISCUSSION

We have documented the fine-scale spatial structure of foliar chemical traits and neutral genetic variation in *Eucalyptus melliodora*. We will now consider the implications of spatial structure for the behavior of various herbivores and for the evolution of foliar
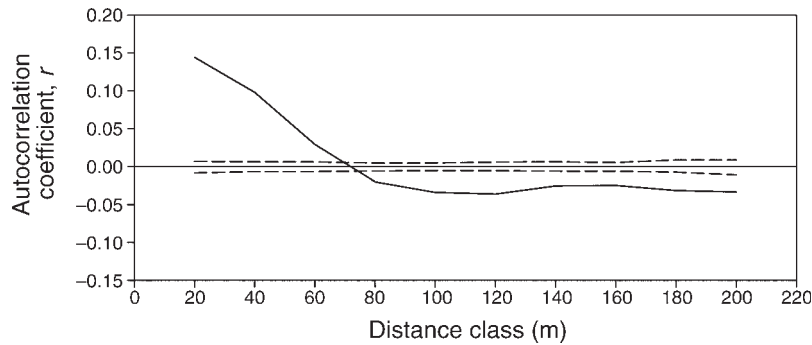
FIG. 5. Global spatial autocorrelation of microsatellite data in 232 *E. melliodora* trees. The correlogram (solid line) is shown with the boundaries of the 95% CI (dashed lines) about the null hypothesis of no spatial structure, estimated by permutations. Distance classes of 20 m were used.

defenses in *Eucalyptus*. Our results suggest that the spatial distribution of genetic and phenotypic variation could influence both the selective pressure imposed by herbivores on eucalypt defenses and the potential of populations to respond to natural selection.

Spatial structure of foliar defenses

Although many experiments on associational aspects of herbivory consider palatable or unpalatable species, intraspecific variation in the palatability of plants also can be considered in a similar framework. Investigation of the spatial distribution of plant phenotypes in nature is clearly essential to understanding the foraging
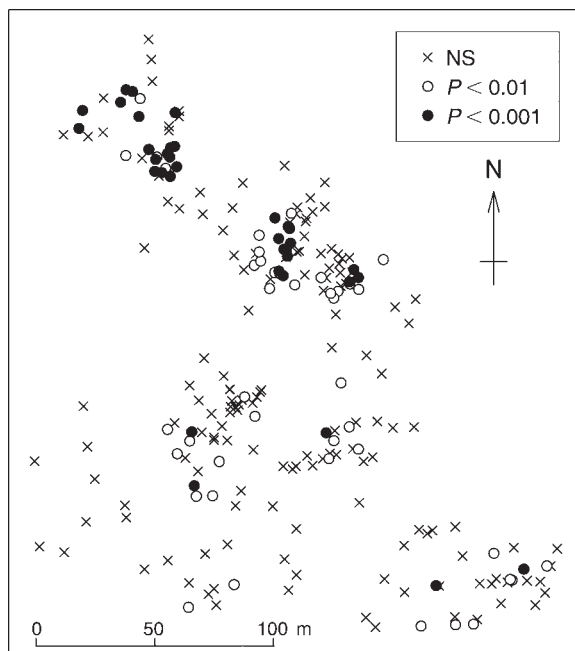

FIG. 6. Two-dimensional local spatial autocorrelation analysis of microsatellite data using six nearest neighbors. Symbols represent the probability of obtaining a higher autocorrelation coefficient under the null hypothesis of no spatial structure, estimated by permutations.

landscapes of herbivores. Our results suggest the potential for associational effects on feeding choices in our study population: significant spatial autocorrelation occurred at a scale relevant to the foraging behavior of important herbivores of *Eucalyptus*.

There are many specialist and generalist herbivores of *Eucalyptus*, including both mammals and invertebrates. Eucalypts produce a broad range of chemical defenses, including tannins, cyanogenic glycosides, terpenoids, and formyl-phloroglucinol compounds (FPCs), which vary in their effectiveness against different herbivores. For instance, the common ringtail possum (*Pseudocheirus peregrinus*) is highly susceptible to FPCs, but highly tolerant of tannins. Common brushtail possums (*Trichosurus vulpecula*), on the other hand, can tolerate more FPCs, but are more susceptible to tannins, than ringtail possums (Marsh et al. 2003).

Common brushtail possums have home ranges of ~1–11 ha in *Eucalyptus* woodland (Kerle 1984). The spatial autocorrelation of foliar concentration of sideroxylonal and the PEG-binding capacity of leaves (the two major determinants of brushtail possum feeding) suggests that an animal would have to travel ~50–80 m to be assured of finding phenotypically uncorrelated trees. Although only a small proportion of the available trees is used for foraging each night, a possum would nevertheless be able to choose between stands of trees with different

TABLE 2. Summary of residual maximum likelihood (REML) model predicting daily dry matter intake (DMI) of *E. melliodora* by common brushtail possums.

Term	Wald statistic	P (χ^2 test)	Coefficient
Experiment	5.93	0.015	8.46
Sideroxylonal	99.90	<0.001	−0.84
PEG-binding capacity	26.74	<0.001	−3.20
Constant			27.72

Notes: Data were collected in two experiments using different possums, which were included as a random effect (for details, see *Methods: Integrating chemical traits to predict feeding by common brushtail possums*). Sideroxylonal and PEG-binding capacity were measured as mg/g dry matter, and DMI was in g dry matter/d. For each chemical trait, df = 1.
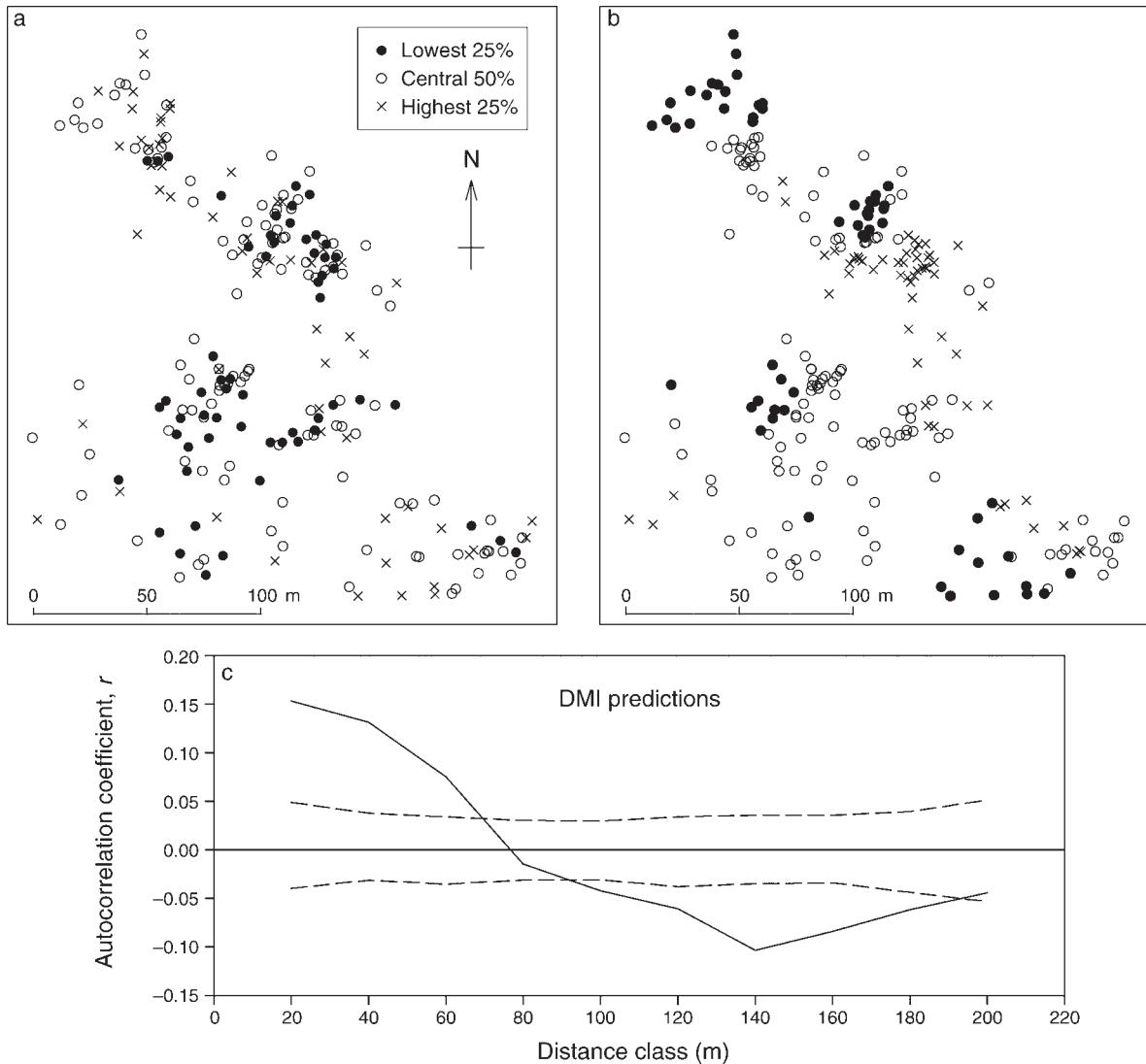
FIG. 7. Maps displaying predicted percentiles for (a) the potential daily dry matter intake (DMI) of *E. melliodora* foliage by common brushtail possums and (b) standard deviation. Symbol shades represent percentiles. On each map the lowest 25% of values are depicted as solid circles, values in the interquartile range (the 50% of values nearest to the median) are shown as open circles, and the highest 25% of values are represented by crosses. (c) The spatial autocorrelogram, with the boundaries of the 95% CI around the null hypothesis of no spatial structure, estimated by permutations (dashed lines).

defense phenotypes within its home range. However, there is also potential for finer-scale processes, such as neighbor contrast effects, because substantial phenotypic variation occurs within patches. Although autocorrelation at a fine scale is strong for phenotypic traits, it only accounts for a proportion of the overall variation.

Differences in the scale of foraging choices are likely to occur among the herbivores of *Eucalyptus*. Sawfly larvae (e.g., *Perga affinis*) move between trees only rarely, whereas Christmas beetles (*Anoplognathus* spp.) can readily fly between neighboring trees (R. L. Andrew, *personal observation*). The latter may be more likely to choose among neighboring trees, whereas vertebrates such as possums are more capable of selecting patches of

less defended trees. However, a possum has sufficient time during its life span to test trees individually, and so may be more capable of selecting the most palatable trees available. Christmas beetles, on the other hand, survive only for a few weeks as adults (Carne 1956), so that the most palatable trees may not be detected and association with unpalatable trees may provide some defense against these herbivores.

Many herbivores are affected by several members of the suite of foliar defenses in eucalypts. The co-distribution of these chemicals is thus important to the foraging environment of a herbivore. To explore this, we predicted the potential palatability of each tree to brushtail possums (*Trichosurus vulpecula*). The predic-

tions of daily potential dry matter intake based on a combination of chemicals characteristics displayed strikingly different spatial patterns to those of single chemicals. Due to the lack of correlation between sideroxylonal and PEG-binding capacity, the areas of the population predicted to be the most palatable are not necessarily those with the lowest concentration of sideroxylonal or the lowest PEG-binding capacity. The mean predicted intake by brushtail possums of *E. melliodora* in the study population (30 g) was approximately half of what they might eat in captivity (Wallis et al. 2002). This prevalence of defended trees may explain why brushtails are generalist herbivores, supplementing the *Eucalyptus* foliage in their diet with understory species.

In addition to leaf chemicals, foraging may be affected by other characteristics of trees. Both koalas and brushtail possums favor larger trees in *Eucalyptus* woodland (Moore and Foley 2005; J. DeGabriel, *personal communication*). This may be because they represent larger food patches, but tree size has a complex relationship with defense. In *E. globulus* and *E. viminalis,* sideroxylonal concentrations, foliar nitrogen, and tree size are intercorrelated (Moore and Foley 2005). However, the correlation of tree size with foliar sideroxylonal is negative in *E. melliodora* in this study, suggesting that larger trees would be preferred on both counts. Decreasing defense with increasing age has been observed before in trees (Boege and Marquis 2005), but natural selection due to the cost of producing sideroxylonal could also produce this pattern. Spatial autocorrelation of phenotypes was weaker for larger trees than for smaller trees, possibly reflecting the accumulation of environmental effects over the long life span of the trees.

Natural selection on foliar defenses due to herbivory

Sideroxylonal offers some protection against several herbivores, including *T. vulpecula*, *P. peregrinus*, the red-bellied pademelon (*Thylogale billardierii*), and Christmas beetles, and as a result is expected to be under selection. Defoliation by both mammalian and insect herbivores is detrimental to growth and survival in young *Eucalyptus* (Raymond 1995, Allcock and Hik 2004) and adult trees may be completely defoliated by either insect and vertebrate herbivores (Martin 1985, Edwards et al. 1993). This must represent a substantial physiological cost, because leaves would normally persist for several years, but it is not known how this affects subsequent reproduction or when the cost is greatest. This is difficult to fully assess in long-lived trees such as *E. melliodora*, as it would require estimation of lifetime reproductive success.

Given that herbivory in *Eucalyptus* must impose some cost, the high variability of foliar sideroxylonal concentration within populations and its significant narrow-sense heritability (Andrew et al. 2005) require explanation. The strength of the selection pressure imposed by

herbivores may be influenced by levels of local variability in palatability, which varies across the population in *E. melliodora*. Spatially heterogeneous selection, which is one possible explanation for high heritability in the face of natural selection, could thus be promoted by the spatial structure of foliar defense variation. Selection on herbivory-related phenotypes can differ in direction and strength with spatial scale in structured populations, i.e., multilevel selection (Aspi et al. 2003). Patch characteristics affect herbivory in a broad range of systems (Hoy et al. 1998, Bergman et al. 2005, Rodriguez-Saona and Thaler 2005), including brushtail possums feeding on different plant species (Pietrzykowski et al. 2003).

Interestingly, the heritability of predicted DMI was marginally significant only when all comparisons were included in the analysis, probably because PEG-binding capacity is not heritable (Andrew et al. 2005). This suggests that the importance of multiple chemical defenses may limit the response of the population to this herbivore.

Spatial structure of genetic variation

The magnitude of spatial genetic autocorrelation that we have detected in *E. melliodora* is among the highest reported for plants (Peakall et al. 2003). This finding is consistent with restricted gene flow, reflecting gravity-dispersed seeds and pollination predominantly due to insects (Potts and Wiltshire 1997). The resulting genetic patches may experience little gene flow, producing overlapping genetic patches that may be capable of responding to natural selection somewhat independently in the short term. The geographic mosaic theory of coevolution (Thompson 1999, Thompson and Cunningham 2002, Rudgers and Strauss 2004) suggests that difference in selection pressures across populations is an important evolutionary mechanism for maintaining genetic variation. This could also occur within continuous plant populations if gene flow is limited at a fine scale and either selection pressures or quantitative genetic parameters also differ at a fine scale. Our results suggest that the first criterion is met in *E. melliodora*, although more research is needed to determine the exact extent of pollen and seed movement.

The patterns of local autocorrelation and phenotypic spatial structure also suggest some ways in which the potential response to selection varies across the population. The processes affecting gene flow and spatial structure in neutral genetic markers will also affect the movement of quantitative trait alleles in the population. The remarkable spatial congruence of sideroxylonal and neutral genetic markers suggests that the genetic variation underlying the sideroxylonal phenotype is similarly structured. The local standard deviation of sideroxylonal content is higher in the southern half of the population, and it is likely that the spatial structure of genetic variance in this trait is also weaker, due to the same combination of dispersal and recruitment that

produced the reduced local spatial genetic autocorrelation. Because the responses of heritable defense traits to selection are determined by the genetic variance present, we predict that fine-scale (e.g., between neighboring trees) herbivore choices will have less impact on the genotypes and phenotypes of future generations in the areas of high local autocorrelation than in those areas with weak or negative autocorrelation.

Literature Cited

Agrawal, A. A., A. Janssen, J. Bruin, M. A. Posthumus, and M. W. Sabelis. 2002. An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores. Ecology Letters 5:377–385.

Allcock, K. G., and D. S. Hik. 2004. Survival, growth, and escape from herbivory are determined by habitat and herbivore species for three Australian woodland plants. Oecologia 138:231–241.

Alm Bergvall, U., and O. Leimar. 2005. Plant secondary compounds and the frequency of food types affect food choice by mammalian herbivores. Ecology 86:2450–2460.

Alm Bergvall, U., P. Rautio, K. Kesti, J. Tuomi, and O. Leimar. 2006. Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. Oecologia 147:253–260.

Andrew, R. L., R. Peakall, I. R. Wallis, J. T. Wood, E. J. Knight, and W. J. Foley. 2005. Can marker-based quantitative genetics in the wild be successful? Heritability and genetic correlation of chemical defences in Eucalyptus. Genetics 171:1989–1998.

Anselin, L. 1995. Local indicators of spatial association: LISA. Geographical Analysis 27:93–115.

Aspi, J., A. Jakalaniemi, J. Tuomi, and P. Siikamaki. 2003. Multilevel phenotypic selection on morphological characters in a metapopulation of Silene tatarica. Evolution 57:509–517.

Baraza, E., R. Zamora, and J. A. Hodar. 2006. Conditional outcomes in plant–herbivore interactions: neighbours matter. Oikos 113:148–156.

Bergman, M., G. R. Iason, and A. J. Hester. 2005. Feeding patterns by roe deer and rabbits on pine, willow and birch in relation to spatial arrangement. Oikos 109:513–520.

Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology and Evolution 20:441–448.

Brenes-Arguedas, T., and P. D. Coley. 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. Oikos 108:410–420.

Cahill, J. F., S. W. Kembel, and D. J. Gustafson. 2005. Differential genetic influences on competitive effect and response in Arabidopsis thaliana. Journal of Ecology 93:958–967.

Cariveau, D., R. E. Irwin, A. K. Brody, L. S. Garcia-Mayeya, and A. von der Ohe. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. Oikos 104:15–26.

Carne, P. B. 1956. A revision of the ruteline genus Anoplognathus Leach (Coleoptera: Scarabaeidae). Australian Journal of Zoology 5:88–144.

Carroll, M. J., A. R. Zangerl, and M. R. Berenbaum. 2000. Heritability estimates for octyl acetate and octyl butyrate in the mature fruit of the wild parsnip. Journal of Heredity 91:68–71.

Covelo, F., and A. Gallardo. 2004. Green and senescent leaf phenolics showed spatial autocorrelation in a Quercus robur population in northwestern Spain. Plant and Soil 259:267–276.

Double, M. C., R. Peakall, N. R. Beck, and A. Cockburn. 2005. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (Malurus cyaneus). Evolution 59:625–635.

Edwards, P. B., W. J. Wanjura, and W. V. Brown. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in Eucalyptus terpenoids. Oecologia 95:551–557.

Geber, M. A., and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. International Journal of Plant Sciences 164:S21–S42.

GenStat. 2005. GenStat Version 8. VSN International, Hemel Hempstead, UK.

Hjältén, J., K. Danell, and P. Lundberg. 1993. Herbivore avoidance by association: vole and hare utilization of woody-plants. Oikos 68:125–131.

Hoy, C. W., G. P. Head, and F. R. Hall. 1998. Spatial heterogeneity and insect adaptation to toxins. Annual Review of Entomology 43:571–594.

Kerle, J. A. 1984. Variation in the ecology of Trichosurus: its adaptive significance. Pages 115–128 in A. S. Smith and I. H. Hume, editors. Possums and gliders. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.

Klinkhamer, P. G. L., T. J. de Jong, and L. A. Linnebank. 2001. Small-scale spatial patterns determine ecological relationships: an experimental example using nectar production rates. Ecology Letters 4:559–567.

Lawler, I. R., W. J. Foley, and B. M. Eschler. 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. Ecology 81:1327–1338.

Lawler, I. R., J. Stapley, W. J. Foley, and B. M. Eschler. 1999. Ecological example of conditioned flavor aversion in plant–herbivore interactions: Effect of terpenes of Eucalyptus leaves on feeding by common ringtail and brushtail possums. Journal of Chemical Ecology 25:401–415.

Legendre, P., and M. J. Fortin. 1989. Spatial pattern and ecological analysis. Vegetatio 80:107–138.

Lynch, M., and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766.

Marsh, K. J., W. J. Foley, A. Cowling, and I. R. Wallis. 2003. Differential susceptibility to Eucalyptus secondary compounds explains feeding by the common ringtail (Pseudocheirus peregrinus) and common brushtail possum (Trichosurus vulpecula). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 173:69–78.

Martin, R. W. 1985. Overbrowsing, and decline of a population of the koala, Phascolarctos cinereus, in Victoria. 1. Food preference and food tree defoliation. Australian Wildlife Research 12:355–365.

Moore, B. D., and W. J. Foley. 2005. Tree use by koalas in a chemically complex landscape. Nature 435:488–490.

Moore, B. D., I. R. Wallis, J. T. Wood, and W. J. Foley. 2004. Foliar nutrition, site quality, and temperature influence foliar chemistry of tallowwood (Eucalyptus microcorys). Ecological Monographs 74:553–568.

Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. Heredity 59:181–197.

Orians, C. M., S. Lower, R. S. Fritz, and B. M. Roche. 2003. The effects of plant genetic variation and soil nutrients on secondary chemistry and growth in a shrubby willow, *Salix sericea*: patterns and constraints on the evolution of resistance traits. Biochemical Systematics and Ecology 31: 233–247.

Peakall, R., M. Ruibal, and D. B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. Evolution 57:1182–1195.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Pietrzykowski, E., C. McArthur, H. Fitzgerald, and A. N. Goodwin. 2003. Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. Journal of Applied Ecology 40:458–469.

Potts, B. M., and R. J. E. Wiltshire. 1997. Eucalypt genetics and genecology. Pages 56–91 *in* J. Williams and J. Woinarshi, editors. Eucalypt ecology: individuals to ecosystems. Cambridge University Press, Cambridge, UK.

Pyke, G. H. 1984. Optimal foraging theory: a critical review. Annual Review of Ecology and Systematics 15:523–575.

Raymond, C. A. 1995. Genetic variation in *Eucalyptus regnans* and *Eucalyptus nitens* for levels of observed defoliation caused by the eucalyptus leaf-beetle, *Chrysophtharta bimaculata* Olivier, in Tasmania. Forest Ecology and Management 72:21–29.

Ritland, K. 1996. A marker-based method for inferences about quantitative inheritance in natural populations. Evolution 50: 1062–1073.

Rodriguez-Saona, C., and J. S. Thaler. 2005. Herbivore-induced responses and patch heterogeneity affect abundance of arthropods on plants. Ecological Entomology 30:156–163.

Rudgers, J. A., and S. Y. Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. Proceedings of the Royal Society 271:2481–2488.

Searle, K. R., N. Thompson Hobbs, and L. A. Shipley. 2005. Should I stay or should I go? Patch departure decisions by herbivores at multiple scales. Oikos 111:417–424.

Smouse, P. E., and R. Peakall. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–573.

Strauss, S. Y., R. E. Irwin, and V. M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. Journal of Ecology 92:132–141.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. American Naturalist 153:S1–S14.

Thompson, J. N., and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. Nature 417:735–738.

Wallis, I. R., M. L. Watson, and W. J. Foley. 2002. Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. Australian Journal of Zoology 50:507–519.