

# The geographical range structure of the holly leaf-miner.

## IV. Effects of variation in host-plant quality

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### Summary

1. Spatial variation and covariation in host-plant quality, herbivore abundance and herbivore mortality were examined across the natural geographical range in Europe of holly *Ilex aquifolium* and the host-specific holly leaf-miner *Phytomyza ilicis*.
2. Although measures of host-plant quality showed substantial between-site variation, no simple spatial pattern in any of the measures (only phosphorus content and leaf mass showed correlations with latitude, longitude or altitude) was detected, and few correlations with tree or local site characteristics.
3. In contrast, measures of the abundance of the leaf-miner exhibited marked spatial patterns, resulting in a lack of simple covariance between leaf-miner abundance and host-plant quality.
4. Different apparent mortalities of the leaf-miner exhibited varied spatial patterns in their intensity, but no evidence of range-wide density dependence, again resulting in few patterns of covariance between intensity of mortality and measures of host-plant quality.
5. The population dynamics of the holly leaf-miner across its geographical range are complex. At any site, the mortality that a population experiences is the sum of largely independent yet spatially structured components, against a background of varying host-plant quality. Despite lacking any marked spatial structure, host-plant quality may have important local effects. These are difficult to detect regionally, and thus may principally contribute noise to regional patterns of levels of oviposition, abundance and mortality.

*Key-words:* abundance, bottom-up factors, geographical range, host-plant quality, macroecology, spatial structure, top-down factors.

*Journal of Animal Ecology* (2004) **73**, 911–924

### Introduction

Insect herbivores are trapped between the low food quality of their host plants and the impact of their natural enemies (Lawton & McNeill 1979). The chemical composition of plants makes them poor food for herbivores: typically they are low in nitrogen but high in lignin and cellulose, and contain secondary metabolites that act as toxins, repellents and digestibility reducers (Hartley & Jones 1997). Meanwhile, predators, parasites and disease reduce herbivore abundance (Hairston, Smith & Slobodkin 1960). How plant quality

and natural enemies interact to limit insect herbivore populations, that is the relative contributions of so-called top-down vs. bottom-up regulatory factors, is a contentious issue (Hunter & Price 1992; Gange & Brown 1997). Experimental tests of the relative strengths of top-down and bottom-up forces remain relatively rare in terrestrial systems (Walker & Jones 2001). Furthermore, most efforts to quantify these two factors measure herbivore abundances only over relatively local scales. This may be highly misleading, given that although most insect herbivores have *relatively* small geographical ranges compared to the more widespread species (Gaston 2003), in *absolute* terms many have moderate to large ranges, over which substantial variation in both plant quality and pressure from natural enemies can be expected. The levels of many plant secondary compounds are altered markedly by abiotic

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environmental factors such as soil type, light, temperature, ultraviolet radiation, ozone and rainfall, as well as being affected by plant growth rate (Herms & Mattson 1992; Jones & Hartley 1999). Thus, it seems highly likely that the quality of a particular food plant for a given species of insect herbivore will differ, perhaps substantially, between, for instance, widely separated parts of its geographical range. Similarly, the species and abundance composition of the assemblages of natural enemies that attack a particular species of insect herbivore changes in space, often also in part as a consequence of environmental factors, which may result in geographical variation in the mortality pressure that they exert (e.g. Schönrogge, Walker & Crawley 1998, 2000; Brewer & Gaston 2003; Gaston 2003).

Spatial variation in the food quality of host plants, in natural enemy pressure and in the interaction between the two seem likely to shape the spatial abundance structure of insect herbivores throughout their geographical ranges. Indeed, such variation probably contributes to the constraints on herbivore range limits, which almost invariably means that even host-specialists may only occupy a small proportion of the geographical ranges of their food plants (e.g. Strong, Lawton & Southwood 1984; Quinn, Gaston & Roy 1997, 1998; Cowley *et al.* 1999; Ungerer, Ayres & Lombardero 1999; for a review see Gaston 2003).

Exploring issues of geographical range structure and its determinants is complicated for most species by many practical considerations, including issues of the spatial extent of sampling required, and the difficulties of measuring resource use, abundances and vital rates. One study system that has proved to be of immense value in examining spatial variation in population processes for a herbivore, at a spectrum of spatial scales, is that of European (or English) holly *Ilex aquifolium* L. (Aquifoliaceae), the host-specialist holly leaf-miner *Phytomyza ilicis* Curtis (Diptera, Agromyzidae) and the suite of natural enemies that attack the leaf-miner (Heads & Lawton 1983; Valladares & Lawton 1991; McGeoch & Gaston 2000; Metcalfe, Marçal & Gaston 2000; Eber *et al.* 2001; Brewer & Gaston 2002, 2003; Klok, Chown & Gaston 2003). In this study, we used this system to test three hypotheses: (i) host-plant quality exhibits systematic patterns of spatial variation across its geographical range; (ii) the distribution and abundance of an insect herbivore is affected by this variation in plant quality; and (iii) the effects of top-down (bird and parasitism attack rates) and bottom-up factors (host-plant quality) on insect herbivore abundance and performance alter across the host plant's geographical range.

#### THE STUDY SYSTEM

Holly is a relatively small, dioecious, evergreen tree. It has distinctive, dark green, glossy leaves that are usually spiny with a thick cuticle. Its natural range extends throughout northwestern, central and southern

Europe (Peterken & Lloyd 1967; Hultén & Fries 1986). It can also be found less commonly in scattered localities in parts of North Africa and has been reported as having a narrow band of distribution extending into Asia Minor, although there is some doubt as to whether the latter is indeed so.

The holly leaf-miner is the most common insect herbivore of European holly. It is strictly monophagous, so its geographical range is ultimately limited by the availability of holly trees. The life histories of *P. ilicis* and its natural enemies have been described in detail by Cameron (1939), Lewis & Taylor (1967) and Ellis (2000). Put briefly, the holly leaf-miner exhibits a univoltine life cycle. In Britain, eggs are laid in June on new holly leaves (the tree has just one flush of new leaves per year) into the base of the underside of the midrib. When laying an egg, the adult female *P. ilicis* leaves a characteristic leaf scar due to the insertion of her ovipositor. The presence of these scars means that oviposition density for a local population may easily be censused. The vast majority of the life history of each individual is spent inhabiting a single holly leaf. After hatching, the larvae eat through the midrib and enter the outer parenchyma of the leaf lamina during the autumn, causing a blister-type mine. They feed throughout the following winter months and pupate in the mine in March, emerging from the leaf as adults in late May or June. The short life span of the adults and the tight synchronization between their emergence and the flush of new leaves severely restricts the possible influence of long-distance immigration and emigration on local population dynamics.

During the period spent within holly leaves, a leaf-miner population may be subject to a number of potential mortalities that are largely sequential, albeit with some overlap. They have been relatively well documented (Cameron 1939; Lewis & Taylor 1967; Heads & Lawton 1983) and include miscellaneous larval deaths, larval parasitism by the parasitoid *Chrysocharis gemma* Walker (Hymenoptera: Eulophidae), bird predation, pupal parasitism by at least eight species of hymenopteran parasitoids and miscellaneous pupal mortality. These causes of mortality can be identified by dissection of the mine at the end of the life cycle of the leaf-miner. In addition, larvae that successfully complete their development and emerge as adult flies can also be identified from the characteristic emergence holes on the leaf surface. Therefore, the holly leaf-miner has a life cycle that is more suitable than most for measuring a number of components of the basic demographic rates of a local population. The level of oviposition, a number of components of mortality and the rate of successful emergence for a local population in any one year can all be assessed by rapid censusing techniques and dissection of a representative sample of leaves from the holly trees at a site.

Previous studies of this system have shown that the holly leaf-miner (i) occurs predominantly in the more northerly parts of the geographical range of its host

plant, where it tends to be ubiquitous; (ii) has an asymmetrical abundance structure to its geographical range, with high to moderate local densities predominantly running in a band across Europe from southwest to northeast, and declining away from this region; and (iii) experiences individual top-down mortality components that are largely independent of one another, and often exhibit different spatial patterns (Brewer & Gaston 2002, 2003). However, variation in bottom-up factors such as host-plant quality, and its interaction with top-down factors, has not been quantified previously. Here we assess spatial variation in both top-down and bottom-up factors across the range of this herbivore, reporting the findings of a new field study.

## Methods

### FIELD SAMPLING

Between August and November 2000 we conducted a survey of holly at 26 sites distributed from the centre to the periphery of its European range (spanning the vast majority of the natural range). The survey included sites in Spain (where holly reaches a southern limit in mainland Europe), central France (essentially the realized core of its distribution), western Germany and eastern France (where it reaches an eastern limit), Belgium, England, western Ireland (where it reaches its western limit), Scotland, and Norway (where it reaches its extreme northern limit) (Fig. 1a). Sampling began at southerly sites and progressed northwards, to minimize seasonal effects, such that the stages of host-plant and leaf-miner development (leaf flush, in the former case) remained broadly constant.

Throughout its range, holly naturally occurs predominantly as a subordinate or understorey plant (except in Norway where it is a canopy species) of deciduous (or mixed) woodland. It was always sampled in this habitat type, which served particularly to avoid the confounding effects of planted holly in heavily human modified environments (e.g. cemeteries, urbanized areas); holly trees in heavily urbanized areas, for example, typically support higher mine densities than those in deciduous woodland (Brewer 2000).

Typically five holly trees (six/seven on occasion) were sampled at each site. For each tree, measurements were taken of (i) soil pH, taken from three amalgamated soil samples from the rooting zone *c.* 1 m from holly base; (ii) density of holly trees (> 20 cm at breast height) within a 20 m radius; (iii) mean leaf production by tree (number of leaves) in previous year for five randomly chosen shoots; and (iv) height of tree (m).

Following Heads & Lawton (1983) and Valladares & Lawton (1991), holly leaf-miner density was estimated on each tree by haphazardly sampling 200 1-year-old leaves (or all the leaves of this age if less than 200 were present on the tree) from all around the canopy, between ground level and a height of approximately 2 m. This protocol gives an estimate of density for the

previous growing season. Leaves of a suitable age can be distinguished readily by their position between the annual nodes on the branch. For each leaf, the number of mines present was recorded, although typically only one mine per leaf is found except in areas of relatively high leaf-miner density (Heads & Lawton 1983), and the number of oviposition scars.

When mines were found, a haphazardly chosen sample of approximately 50 mined 1-year-old leaves was removed from each tree. If 50 mines could not be found on a particular tree, all the visible mines of the appropriate age were removed. These mines were dissected to establish the number of holly leaf-miner individuals that were subject to the different mortalities (miscellaneous larval mortality, larval parasitism, bird predation, pupal parasitism, miscellaneous pupal mortality) or emerged successfully as adults, following the procedures given by Heads & Lawton (1983).

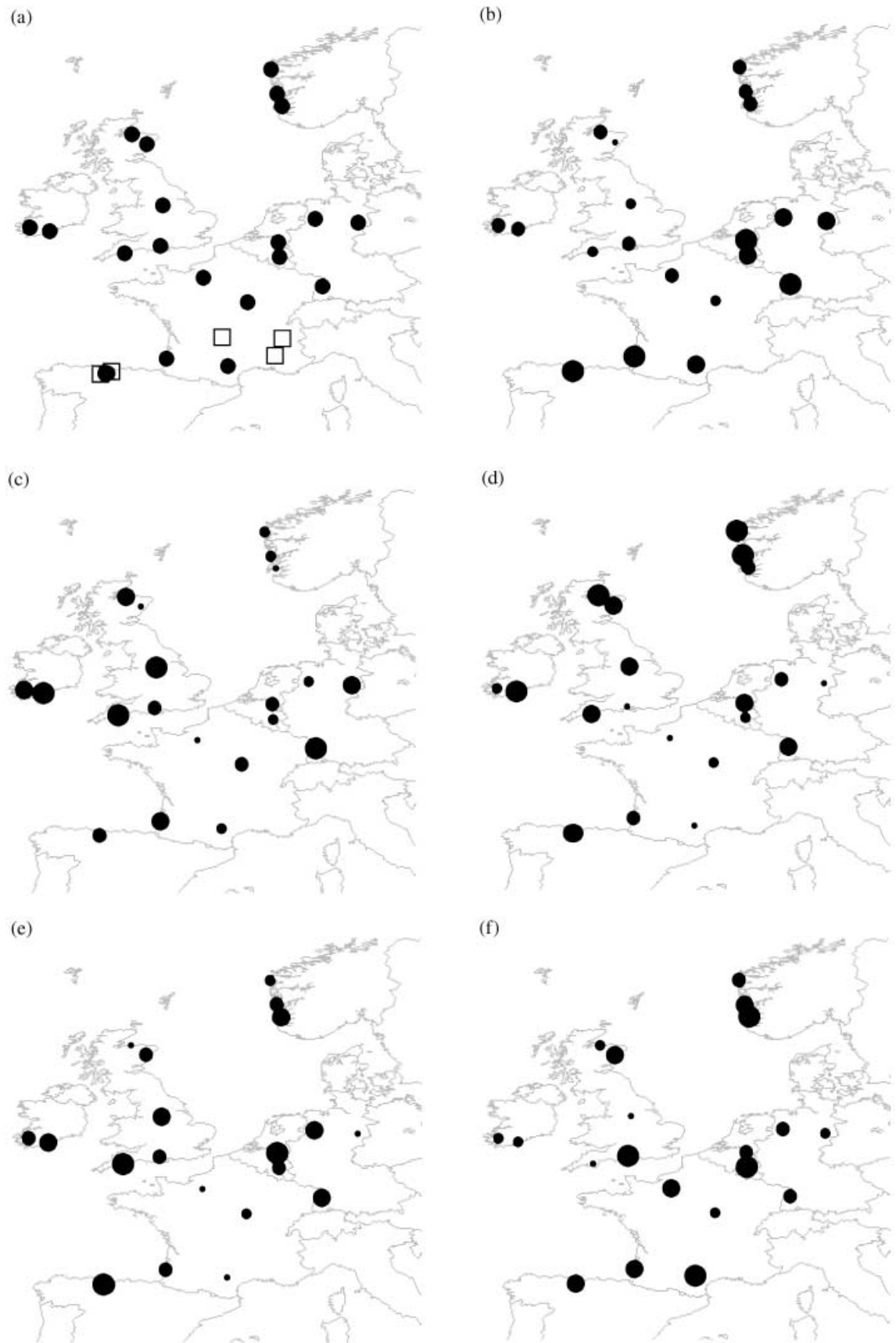
Where available, 10 leaves of each of five types [mature (previous year's cohort; one year-old) unmined by leaf-miner, mature with leaf-miner oviposition scar but unmined, mature mined, young (sample year's cohort) unmined, young mined] were collected in a haphazardly chosen sample from each tree, weighed and then dried for chemical analysis.

### HOST-PLANT QUALITY

On returning to the laboratory, leaf samples were dried to constant mass in an oven at 80 °C and reweighed. Water content was determined from the mass loss. The dry plant material was milled and analysed for total nitrogen content using a continuous flow colourimetric autoanalyser (Segmented Flow Autoanalyser, Burkard Scientific, Uxbridge, UK), following wet acid digestion (Allen 1989). Nitrogen content was measured as ammonium by a modified Bertholet reaction and phosphorus content by the molybdenum blue method (Hinds & Lowe 1980; Rowland 1983; Allen 1989), and the results were expressed as percentage of dry weight. Phenolic content was measured by the Folin-Ciocalteu method using tannic acid as a standard, after extraction of dried milled leaf material in boiling aqueous 50% methanol (following Kerlake, Woodin & Hartley 1998). Phenolic content was expressed as percentage of dry weight (calculated as mg tannic acid equivalents per mg leaf mass  $\times$  100).

Given the large quantities of material collected and the limited time and resources for chemical analyses, samples were not analysed from the five sites that were likely (given their position relative to other sites) to provide least information about spatial variation in holly quality (two sites in Spain and three sites in France; see Fig. 1a).

A total of 136 holly trees were sampled during the course of the survey. This resulted in the examination of 26 602 leaves, and the collection of over 5500 leaves for chemical analysis. The results presented below are based on site means for the different variables.



**Fig. 1.** Maps of (a) the distribution of sample sites (solid symbols, host-plant quality determined; open symbols, host-plant quality not determined), and (b) leaf mass, (c) water content, (d) phosphorus content, (e) nitrogen content and (f) phenolic content for mature mined leaves (with symbols scaled according to the value of the variable in each case).

## DATA ANALYSIS

*The demographic data sets*

Because the mortalities acting on the holly leaf-miner are largely sequential in this system, the number of mines that can potentially succumb to a particular mortality is the total number of mines minus the number killed by previous mortalities. This smaller proportion of mines is used to calculate *apparent* mortality (Bellows, Van Driesche & Elkinton 1992) as opposed to *real* mortality (which is the proportion of all mines). In all the analyses below, apparent mortalities were used unless stated otherwise. These were calculated as the total number of each mortality recorded divided by the number of mines available to that mortality across all trees that were sampled at a site. Oviposition rate was measured as the total number of egg scars divided by the number of leaves examined during density estimation at each site. Successful emergence was measured as the proportion of the total number of leaf-miners collected that had emerged successfully as adults, as it will always be 100% of the mines that have not succumbed to any previous mortality (Brewer & Gaston 2003). A measure of the density of successful emergences (the number of adults produced) was also generated by expressing these relative to the density of mined leaves.

Values for a number of variables were transformed to meet the assumptions of parametric statistical tests where these were employed: mine density, proportion of successful emergences, density of successful emergences, and all mortality rates were arcsin<sup>1/2</sup> transformed; altitude and soil pH were logarithmically transformed; and oviposition density was square root transformed.

*Spatial autocorrelation*

Spatial autocorrelation analysis (Cliff & Ord 1973; see also Legendre & Fortin 1989) was used to characterize the spatial structure of host-plant quality, and the abundance and the demographic rates of the holly leaf-miner. Moran's I was calculated for five equal distance intervals, and spatial correlograms were produced and

tested for significant spatial dependence. As the survey covered a relatively large geographical area, site coordinates (measured as decimal degrees longitude and latitude) were not treated as Cartesian coordinates when measuring distances between them. Instead, distances along great circles were calculated to take into account the curvature of the Earth's surface. Bonferroni's correction for multiple comparisons was used when assessing overall correlogram significance.

The relatively low number of data points, resulting from practical constraints on the field work and chemical analyses, prevented explicit controlling for spatial structure of variables in bivariate and multivariate analyses (see Brewer & Gaston 2002, 2003). However, this was not particularly problematic, given the lack of strong simple spatial structuring of many of the variables considered and the diversity of forms this took where it did occur (see below).

**Results**

## HOST-PLANT QUALITY

The same pattern of variation in host-plant quality across leaf types was observed at all sites. Pooling data across sites there were significant differences between the measures of host-plant quality between leaf types, but these were largely due to leaf age rather than exploitation by miners (Table 1). Thus, the young leaves were heavier and had higher water, phosphorus and nitrogen content than mature leaves (ANOVA for effect of leaf type;  $P < 0.001$  for all parameters), but mined and unmined leaves did not differ in these parameters within a given age class (Tukey's multiple comparison test;  $P > 0.05$  in all cases). The only exception to this was for phenolic levels: younger leaves had lower levels than mature ones, but mature leaves attacked by holly leaf-miners, whether it led to a mine or remained just as an oviposition scar, had elevated phenolic levels relative to unmined mature leaves. This association with damage was much less pronounced in young leaves, hence the phenolic levels in mined and unmined leaves did not differ (Table 1; Tukey's multiple comparison test: mature mined = mature, oviposition scar and unmined > mature unmined > young mined = young unmined).

**Table 1.** The fresh weight (g) of five different classes of mined and unmined holly leaves and their water (%), phosphorus (% dry weight), nitrogen (% dry weight) and phenolic (% dry weight) contents. Within each of the measured parameters, mean values followed by superscript letters differ significantly (ANOVA; Tukey's multiple comparisons,  $P < 0.05$ )

	Leaf mass		Water		Phosphorus		Nitrogen		Phenolic mean	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mature unmined	10.731 <sup>a</sup>	1.271	63.65 <sup>a</sup>	2.671	0.0832 <sup>a</sup>	0.015	1.402 <sup>a</sup>	0.185	1.919 <sup>a</sup>	0.573
Mature, oviposition scar and unmined	11.022 <sup>a</sup>	1.953	63.89 <sup>a</sup>	3.017	0.0829 <sup>a</sup>	0.015	1.436 <sup>a</sup>	0.156	2.531 <sup>b</sup>	0.648
Mature mined	10.441 <sup>a</sup>	1.779	64.42 <sup>a</sup>	2.797	0.0864 <sup>a</sup>	0.011	1.421 <sup>a</sup>	0.127	2.624 <sup>b</sup>	0.473
Young unmined	12.589 <sup>b</sup>	1.933	67.07 <sup>b</sup>	3.523	0.1237 <sup>b</sup>	0.024	1.649 <sup>b</sup>	0.228	1.244 <sup>c</sup>	0.552
Young mined	12.676 <sup>b</sup>	1.919	67.32 <sup>b</sup>	3.487	0.1232 <sup>b</sup>	0.026	1.648 <sup>b</sup>	0.252	1.296 <sup>c</sup>	0.551

**Table 2.** Spearman's rank correlations across sites between (a) leaf mass, (b) water, (c) phosphorus, (d) nitrogen, and (e) phenolic content of five different kinds of holly leaves. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Sample sizes in parentheses

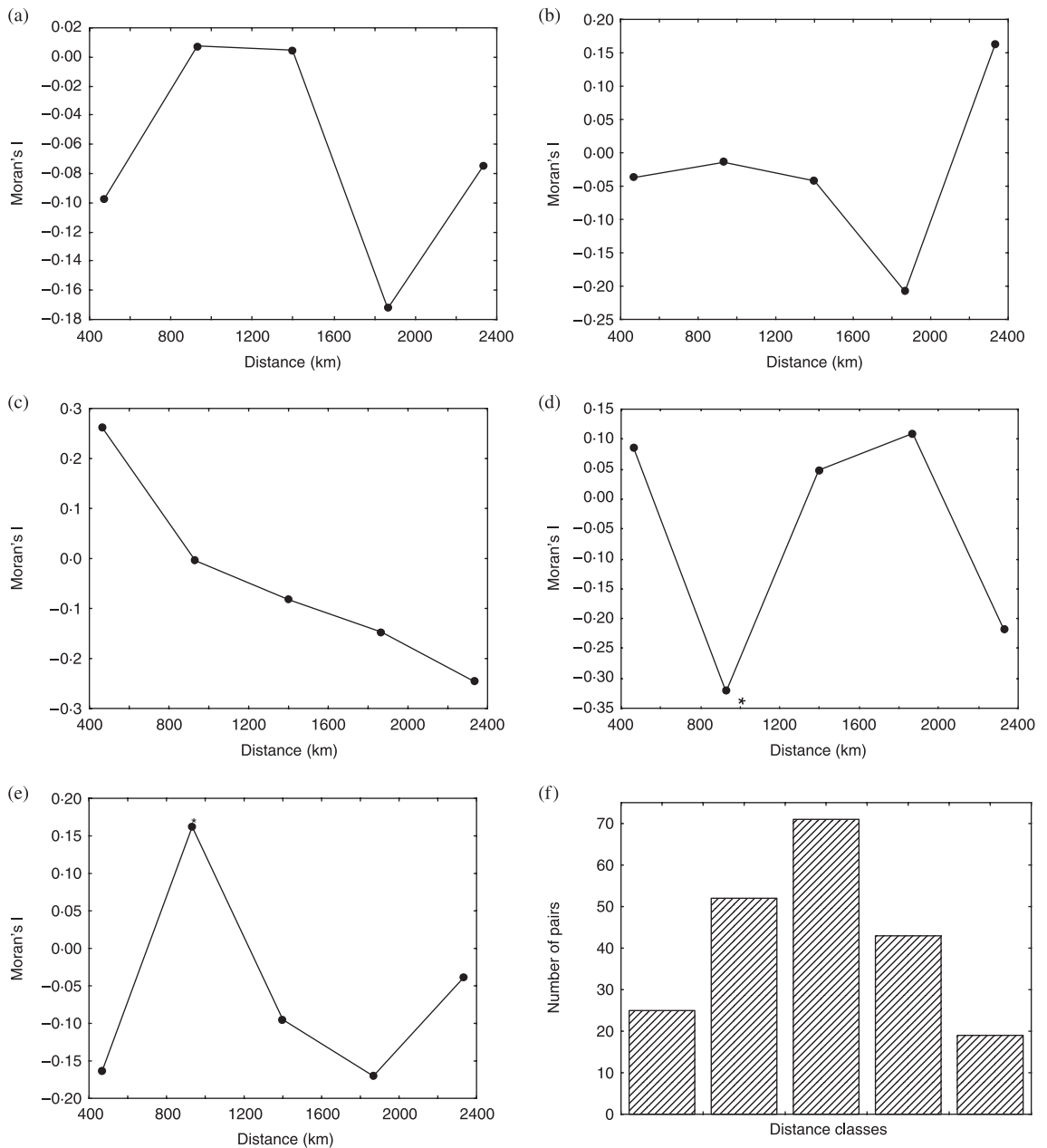
	Mature unmined	Mature, oviposition scar and unmined	Mature mined	Young unmined
(a) Leaf mass				
Mature, oviposition scar and unmined	0.022 (21)			
Mature mined	-0.114 (21)	0.332 (21)		
Young unmined	0.365 (21)	-0.325 (21)	-0.404 (21)	
Young mined	-0.208 (21)	0.123 (21)	0.140 (21)	-0.197 (21)
(b) Water				
Mature, oviposition scar and unmined	0.931 (21)***			
Mature mined	0.878 (21)***	0.870 (21)***		
Young unmined	0.750 (21)***	0.724 (21)***	0.709 (21)***	
Young mined	0.731 (21)***	0.722 (21)***	0.716 (21)***	0.943 (21)***
(c) Phosphorus				
Mature, oviposition scar and unmined	0.588 (20)**			
Mature mined	0.810 (21)***	0.712 (20)***		
Young unmined	0.579 (23)***	0.537 (20)*	0.440 (21)*	
Young mined	0.724 (21)**	0.482 (20)*	0.671 (21)***	0.761 (21)**
(d) Nitrogen				
Mature, oviposition scar and unmined	0.542 (20)**			
Mature mined	0.776 (21)***	0.359 (20)		
Young unmined	0.674 (23)***	0.694 (20)***	0.402 (21)	
Young mined	0.719 (21)***	0.431 (20)	0.582 (21)**	0.654 (21)**
(e) Phenolics				
Mature, oviposition scar and unmined	0.850 (13)***			
Mature mined	0.828 (21)***	0.806 (13)***		
Young unmined	0.511 (23)*	0.646 (13)*	0.650 (21)**	
Young mined	0.438 (21)*	0.479 (13)	0.652 (21)**	0.957 (21)***

With the notable exception of leaf mass, the measures of host-plant quality typically showed marked and highly significant across-site correlations between the five different types of leaves (Table 2). That is, differences between sites in a measure of host-plant quality for one leaf type also tended to be reflected in other leaf types. Thus, in the main, here we will concentrate on the findings for the mature mined leaves (i.e. those which had been exploited by the leaf-miner, and thus whose quality should be most relevant). For the mature mined leaves, there was considerable between-site variation in the different measures: leaf mass 0.54–1.32 g, water content 58.35–68.18 (% dry wt), phosphorus content 0.06–0.11 (% dry wt), nitrogen content 1.18–1.70 (% dry wt) and phenolic content 1.50–3.27 (% dry wt).

The correlograms showed a lack of significant spatial dependence in all of the measures of host-plant quality, although values of Moran's I declined systematically with longer distances for phosphorus content, showing that trees become less similar between sites that are further apart (Fig. 2). While concentrating on values of Moran's I based on more than 30 pairwise comparisons (which will be more robust), most measures of host-plant quality showed a decline in similarity with distance; almost invariably values of Moran's I were consistently low, indicating little similarity in host-plant quality between sites. In a related vein, none of the measures of local plant quality exhibited signi-

ficant correlations with the latitude, longitude or altitude of sample sites, with the exception of fresh leaf mass which increased with longitude (i.e. towards the east;  $r_s = 0.470$ ,  $n = 21$ ,  $P < 0.05$ ), and phosphorus content which increased with latitude ( $r_s = 0.483$ ,  $n = 21$ ,  $P < 0.05$ ) and showed a decline with altitude that bordered on statistical significance ( $r_s = -0.426$ ,  $n = 21$ ,  $P = 0.054$ ) (see also Fig. 1b–f).

There were also few correlations between measures of host-plant quality and other features of the trees or the local environment that were statistically significant or close to so being, except for a decline in water content ( $r_s = -0.464$ ,  $n = 21$ ,  $P < 0.05$ ) and phenolic content ( $r_s = -0.427$ ,  $n = 21$ ,  $P = 0.054$ ) with soil pH, an increase in phosphorus content with leaf production ( $r_s = 0.432$ ,  $n = 21$ ,  $P = 0.0503$ ), and an increase in phosphorus content ( $r_s = 0.595$ ,  $n = 21$ ,  $P < 0.01$ ) and phenolic content ( $r_s = 0.526$ ,  $n = 21$ ,  $P < 0.05$ ) with tree height. The increase in phenolic content with tree height probably reflects an increase in tree age with height. These relationships tended, moreover, to be associated with patterns of spatial variation in host-plant quality, with an increase in leaf production with latitude ( $r_s = 0.404$ ,  $n = 26$ ,  $P < 0.05$ ) and decrease with altitude ( $r_s = -0.549$ ,  $n = 26$ ,  $P < 0.01$ ) and tree height increases with latitude ( $r_s = 0.518$ ,  $n = 26$ ,  $P < 0.01$ ) and decreases with altitude ( $r_s = -0.467$ ,  $n = 26$ ,  $P < 0.05$ ); tree height also tends to decline with longitude ( $r_s = -0.420$ ,  $n = 26$ ,  $P < 0.05$ ).



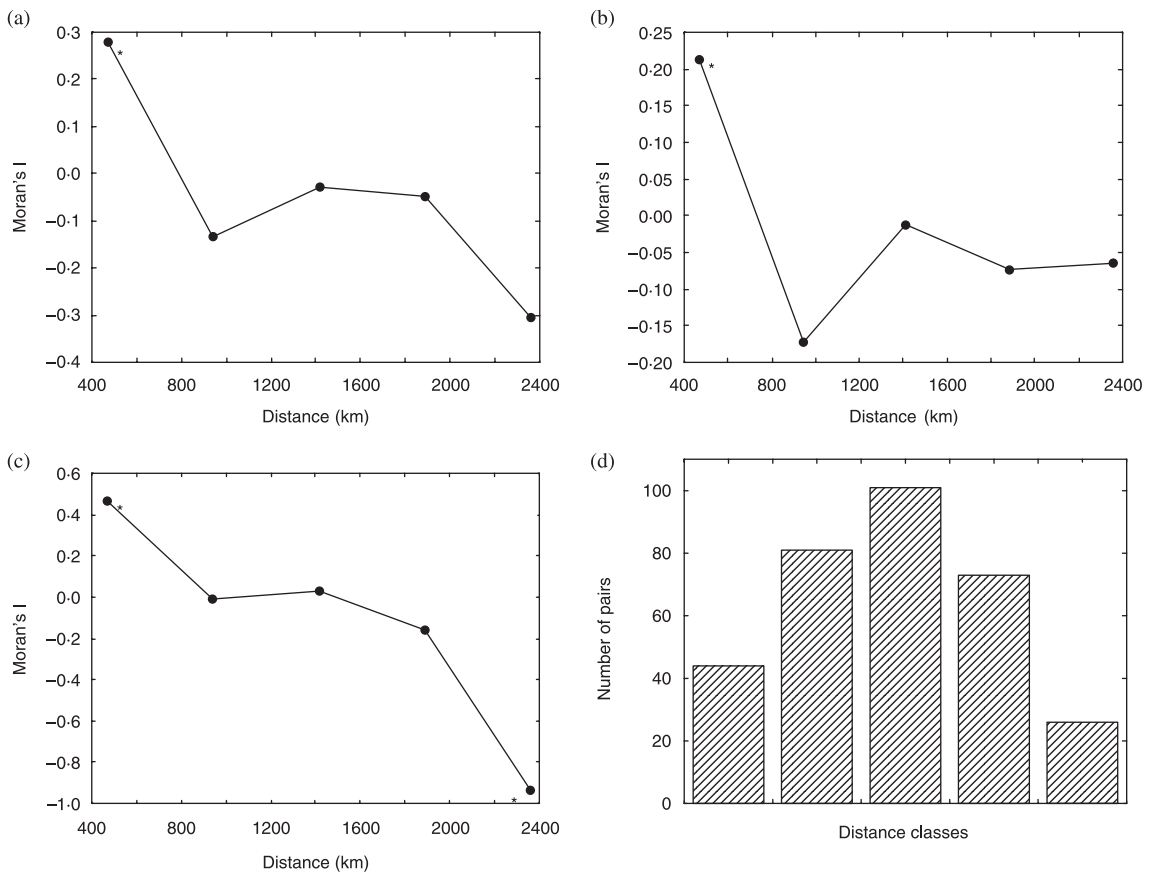
**Fig. 2.** Spatial correlograms of the holly host-plant quality across its geographical range for mature mined leaves. Scale on the  $x$ -axis represents the maximum in each distance class. Asterisks indicate significant values for Moran's  $I$  ( $P < 0.05$ ). In no case was there overall correlogram significance, tested using Bonferroni's correction for multiple comparisons. (a) leaf mass, (b) water content, (c) phosphorus content, (d) nitrogen content, (e) phenolic content and (f) the number of pairs of comparisons within each distance class for the autocorrelation.

#### HOST-PLANT QUALITY AND HERBIVORE ABUNDANCE

There was evidence of spatial structure to measures of holly leaf-miner abundance. Correlograms of the density of oviposition (eggs), larvae, and successful emergences all exhibited highest autocorrelations at the shortest lag distances, and a tendency for autocorrelations to decline with longer lags (i.e. densities were more similar at sites closer together; Fig. 3). Similarly, the density of successful emergences increased with

longitude ( $r_s = 0.405$ ,  $n = 26$ ,  $P < 0.05$ ), and the densities of oviposition, larvae and successful emergences all increased with latitude and decreased with altitude (Fig. 4).

The lack of spatial structure in host-plant quality and its existence in leaf-miner abundance meant that there was no simple covariance between the two. Oviposition density, larval density and the density of emerging adults were all uncorrelated with any of the measures of host-plant quality as measured using mature mined leaves.



**Fig. 3.** Spatial correlograms of holly leaf-miner abundance from across its geographical range. Scale on the x-axis represents the maximum in each distance class. Asterisks indicate significant values for Moran's I ( $P < 0.05$ ). Overall correlogram significance was tested using Bonferroni's correction for multiple comparisons. (a) oviposition density (NS), (b) larval density (NS), (c) successful emergence density ( $P < 0.001$ ) and (d) the number of pairs of comparisons within each distance class for the autocorrelation.

#### HOST-PLANT QUALITY AND HERBIVORE MORTALITY

Correlograms revealed varied patterns of spatial structure to the different mortalities influencing the holly leaf-miner (Fig. 5). Miscellaneous larval mortality exhibited a decline in Moran's I toward intermediate distances and a slight subsequent increase, but no overall significant pattern (Fig. 5a). Larval parasitism showed a significant pattern of decline in Moran's I with longer distances (Fig. 5b). Bird predation showed a hump-shaped pattern, with significant autocorrelations at longer distances (Fig. 5c), pupal mortality showed a U-shaped pattern and miscellaneous pupal mortality showed the highest autocorrelation at long distances (Fig. 5e). Successful emergence exhibited a decline in Moran's I with increasing distance (Fig. 5f).

The different mortalities also exhibited some correlations with longitude and latitude, but not altitude, with miscellaneous larval mortality declining with longitude ( $r_s = -0.423$ ,  $n = 24$ ,  $P < 0.05$ ) and larval parasitism declining with longitude ( $r_s = -0.690$ ,  $n = 24$ ,  $P < 0.001$ ) and with latitude ( $r_s = -0.542$ ,  $n = 24$ ,  $P < 0.01$ ). Successful emergence increased with longitude

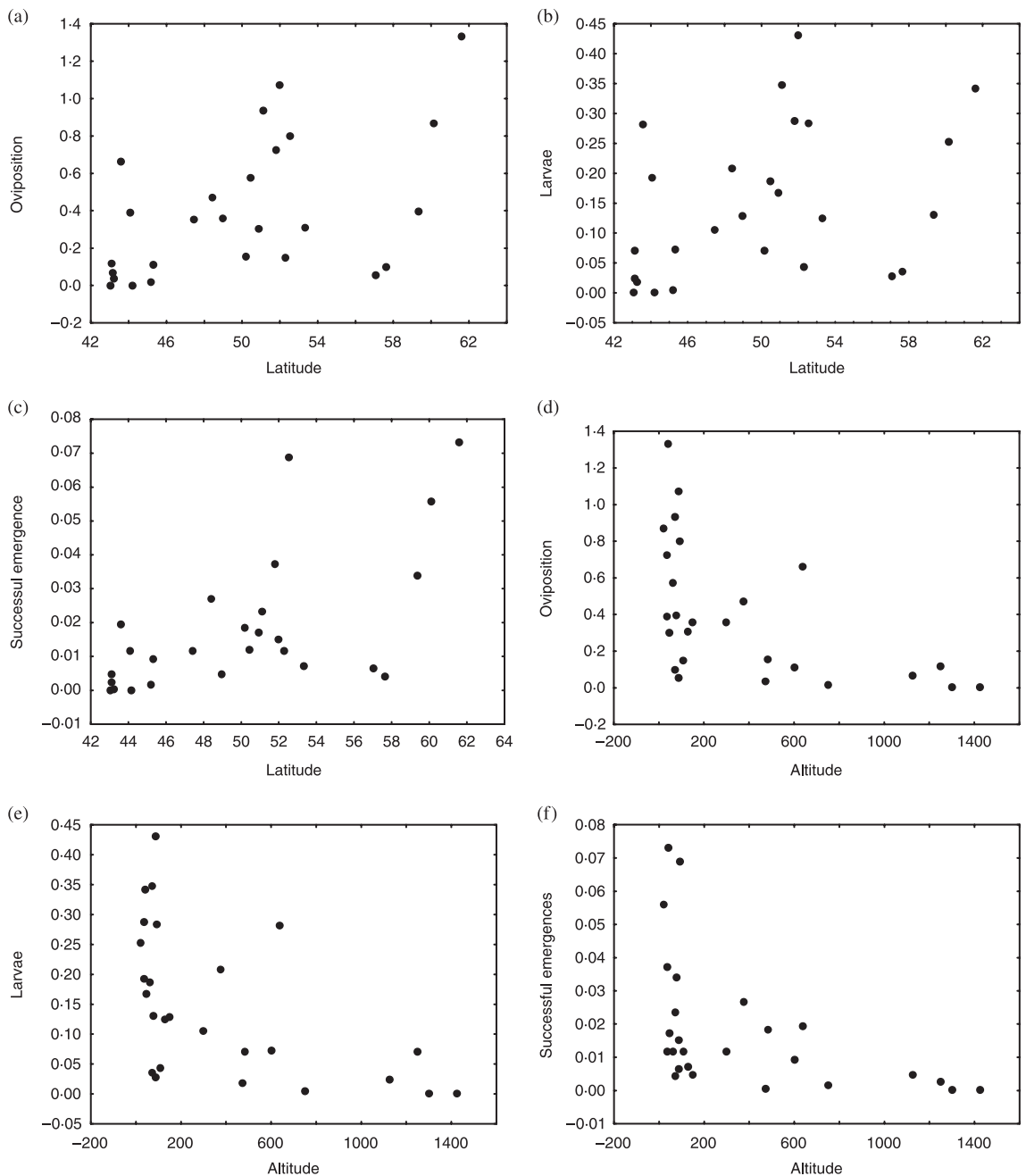
( $r_s = 0.634$ ,  $n = 24$ ,  $P < 0.001$ ) and increased marginally with latitude ( $r_s = 0.383$ ,  $n = 24$ ,  $P = 0.064$ ).

The existence of range-wide density dependence was tested separately for each of the components of leaf-miner mortality. None of the apparent mortalities exhibit statistically significant density dependence.

There were only two significant relationships between a mortality factor and a measure of host-plant quality, a negative relationship between leaf mass and miscellaneous larval mortality ( $r_s = -0.477$ ,  $n = 21$ ,  $P < 0.05$ ) and a positive relationship between leaf nitrogen and the proportion of leaf-miners predated by birds ( $r_s = 0.575$ ,  $n = 21$ ,  $P < 0.01$ ).

#### Discussion

Summarizing conclusions with regard to the three original hypotheses, (i) measures of holly quality as a host plant do exhibit substantial variation between sites across its geographical range, but we found little evidence that this variation takes any marked simple spatial form; (ii) in contrast, holly leaf-miner abundance exhibits marked spatial structure, which means that patterns of variation in host-plant quality and leaf-miner abundance



**Fig. 4.** Relationships between densities of oviposition, mined leaves and successful emergence of the holly leaf-miner and latitude (degrees) (a–c) and altitude (m) (d–f). (a)  $r_s = 0.512$ ,  $P < 0.01$ ; (b)  $r_s = 0.454$ ,  $P < 0.05$ ; (c)  $r_s = 0.603$ ,  $P < 0.01$ ; (d)  $r_s = -0.639$ ,  $P < 0.001$ ; (e)  $r_s = -0.639$ ,  $P < 0.001$ ; (f)  $r_s = -0.631$ ,  $P < 0.001$ ;  $n = 26$  in all cases.

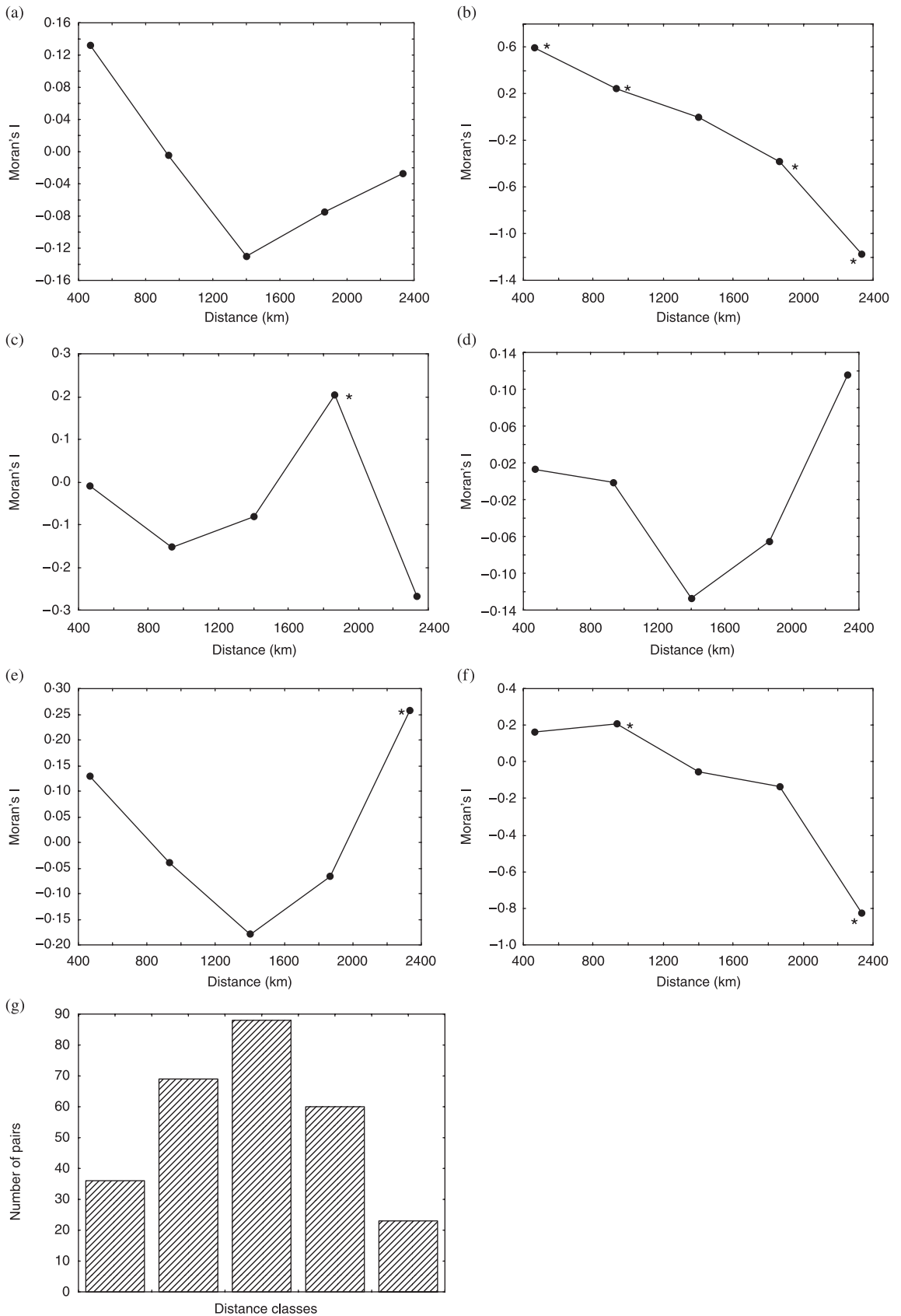
show no simple relationship; and (iii) the different forms of mortality of the leaf-miner also exhibit notable cases of spatial structure, which means that in the main they also do not covary in simple ways either with leaf-miner abundance or with variation in host-plant quality.

#### HOST-PLANT QUALITY

Measures of the quality of holly as a host plant exhibited no simple patterns of spatial variation across its geographical range (Fig. 1). There could be several

reasons for this. First, sample sizes could be insufficient relative to the strength of any patterns. If so, these patterns must be weak compared with those in holly leaf-miner abundances.

Secondly, our measures of host-plant quality may have been inappropriate. Holly is of poor nutritional quality in terms of the criteria known to be important to herbivores, namely water content, nutrient levels and secondary compound content (Hartley & Jones 1997). Indeed, the low water and nitrogen content of holly and its high levels of toughness and phenolic compounds have been suggested as the reason for the



**Fig. 5.** Spatial correlograms of holly leaf-miner apparent mortality and successful emergence from across its geographical range. Scale on the x-axis represents the maximum in each distance class. Asterisks indicate significant values for Moran's I ( $P < 0.05$ ). Overall correlogram significance was tested using Bonferroni's correction for multiple comparisons. (a) miscellaneous larval mortality (NS), (b) larval parasitism ( $P < 0.001$ ), (c) bird predation (NS), (d) pupal parasitism (NS), (e) miscellaneous pupal mortality (NS), (f) successful emergence ( $P < 0.001$ ) and (g) the number of pairs of comparisons within each distance class for the autocorrelation.

prolonged larval development of holly leaf-miners (Potter & Kimmerer 1986; Kimmerer & Potter 1987). It thus seems unlikely that none of our chosen indicators of leaf quality would have had any influence on holly leaf-miner success, particularly when previous work has detected an influence of these parameters at a local scale, albeit in an unexpected direction: higher nitrogen content appears to have a negative impact on the miner (see below; Valladares & Lawton 1991). However, as well as phenolic compounds, holly contains a second class of secondary compounds which we did not measure, namely saponins. These have been suggested to be the major antiherbivore defence of young holly leaves, whereas phenolics defend the more mature leaves (Potter & Kimmerer 1989), so it is at least possible that had we measured saponins we may have found some strong spatial patterns. However, given the lack of such patterns in several other key foliar constituents, this does not seem likely.

Thirdly, although many factors which influence host-plant quality are known to vary with environmental conditions (e.g. light levels, soil type), there may well be no consistent trend in many of these factors with changing latitude or longitude. For example, total solar radiation is less at higher latitudes, but a far bigger effect on the chemical composition of holly at a particular site may result from differences in light availability due to aspect (whether the plants are on a south facing slope) or localized shading by other species. Overall our results suggest that local site-based factors influence plant chemical composition more than geographical scale gradients.

Latitudinal gradients in host quality, at least in terms of defence production, have been detected previously (Levin & York 1978; Coley & Aide 1991), albeit usually over much larger geographical areas than are considered here (but see Brower *et al.* 1972; Kokkini, Karousou & Vokou 1994). In our study any such gradients are certainly not detectable, if they exist at all, possibly because we were considering solely the temperate region and climatic variation may not have been great enough to have a consistent impact in the face of smaller scale variation in other abiotic and biotic factors. Phenotypic plasticity in response to this small-scale environmental variation would explain why individual sites vary so much without contributing to a simple pattern in spatial variation. For example, soil nutrient levels would be an obvious determinant of holly nitrogen content, but both soil nutrients and light levels are known to affect allocation to defence compounds (Bryant, Chapin & Klein 1983; Jones & Hartley 1999; Burns, Gleadow & Woodrow 2002). In addition, populations of holly in different areas of the range will vary markedly in genotype (Linhart & Grant 1996). Genotype (Krischik & Denno 1983; Bowers & Stamp 1993), and genotype–environment interactions (Coley, Bryant & Chapin 1985; Agrawal, Gorski & Tallamy 1999) are both strong influences on plant defence allocation. Lastly, there is often substantial variation

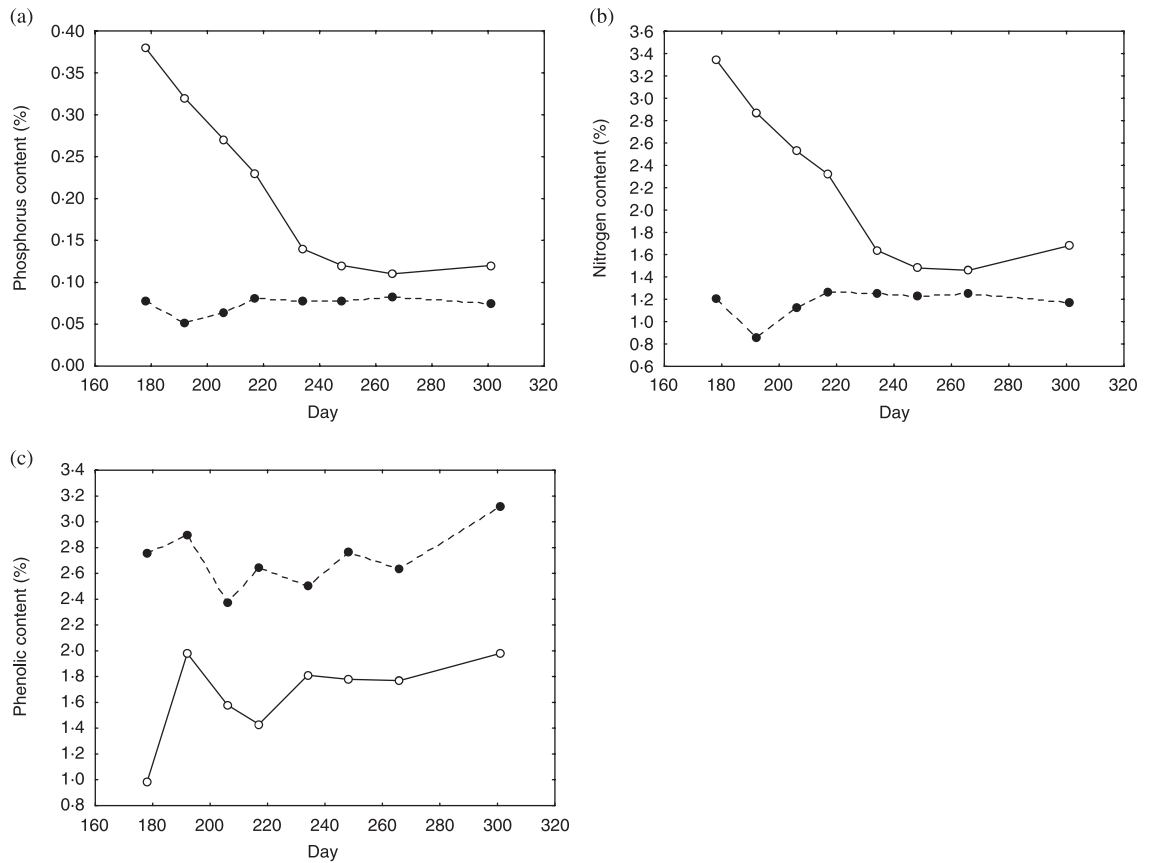
between individuals in host-plant quality (Hartley & Jones 1997). This variation may be extremely small-scale. For example, in birch, variation in leaf quality within trees is even greater than variation between individuals (Suomela, Ossipov & Haukioja 1995). All these factors mean that plant quality is hugely variable at a range of spatial scales, so it is perhaps unsurprising that large scale spatial patterns in plant quality could not be detected in this study. It could contribute none the less to local scale variation in abundances, which can vary markedly between trees, albeit that this variation constitutes variance about broader regional patterns in holly leaf-miner abundance.

Fourthly, temporal variation in host-plant quality could also potentially confound some of the analyses reported, despite attempts to conduct sampling in a geographical sequence that ensured leaves were consistently collected from the different sites at a similar stage in their development and age. However, studies of local temporal variation in the measures of quality have shown that both for young and mature leaves these tended to stabilize at a much earlier stage in the season than that at which the field sampling was conducted (Fig. 6), so this is not an explanation for the lack of regional scale effects.

#### HOST-PLANT QUALITY AND HERBIVORE ABUNDANCE

There is evidence of some simple spatial structure to the large scale geographical variation in the abundance of the holly leaf-miner (Figs 3 and 4), which broadly concurs with the results of a previous field study (Brewer & Gaston 2002). Densities are low in the extreme south (with the range of the leaf-miner not extending as far south as that of holly), and high to moderate densities lie predominantly in a band running northeast to southwest, from the southwest coast of Norway, across Denmark, Holland, Belgium, northern France, southern Britain, and the extreme southwest of Ireland. This is manifested here in the broad increase in density with latitude (Fig. 4). Holly tends to occur at progressively lower elevations with increasing latitude (Brewer & Gaston 2002), which also results in a marked overall correlation between leaf-miner density and altitude (Fig. 4).

Previous work has suggested that much of the spatial variation in holly leaf-miner densities is correlated with spatial variation in ambient climatic conditions, notably temperature and precipitation (or humidity; Brewer & Gaston 2002). A significant association with climatic variation remains even when the spatial structuring of the environment is explicitly accounted for in the analyses. What has remained unclear, however, is to what extent these results follow from a direct effect of climate on the holly leaf-miner itself, or an indirect one through its effects on the quality of holly as a host-plant or on the impact of natural enemies. The lack of any simple association between variation in host-plant



**Fig. 6.** Temporal changes in mean (a) phosphorus, (b) nitrogen and (c) phenolic content of holly leaves from trees at a site in Banchory, Scotland. Days run from 1 January 2000, open circles are young leaves (flushed in 2000), and closed circles are mature leaves. Based on 10 mature and 20 young leaves from five trees.

quality and variation in leaf-miner abundance found in the present study makes it unlikely that the effects of climate are acting through their influence on host-plant quality.

More generally, evidence for a direct influence of host-plant quality on the performance of the holly leaf-miner has remained poor. Thus, among several characteristics of holly bushes at a local scale, Valladares & Lawton (1991) found only a negative correlation between infestation levels and nitrogen levels in young foliage in one generation of flies, and against a background of multiple tests regarded this result as difficult to interpret.

Miscellaneous larval mortalities of the holly leaf-miner, which tend to occur early in the larval stage, have been attributed to a physiological response of the host tree to the presence of the herbivore (e.g. Ellis 2000). However, the discovery that the level of such mortalities tends to covary positively with the levels of larval parasitism suggests that an important contributor may actually be attacks by the larval parasitoid *C. gemma* that perhaps do not result in oviposition or in development of a parasitoid (Brewer & Gaston 2003). A positive relationship between levels of miscellaneous larval mortality and larval parasitism is also exhibited by the data from the present study ( $r_s = 0.502$ ,  $n = 24$ ,  $P < 0.05$ ). The greater proportion of miscellaneous

larval mortalities of the holly leaf-miner are likely to be the result of premature deaths when *C. gemma* larvae are killed by cold snaps in late winter, leaving no immediate evidence that the leaf-miners were parasitized (Klok *et al.* 2003).

#### HOST-PLANT QUALITY AND HERBIVORE MORTALITY

The different forms of mortality of the holly leaf-miner exhibit very different patterns of spatial variation. Despite the smaller sample sizes, the findings of the present study tend to substantiate those of previous work (Brewer & Gaston 2003), suggesting that these patterns are reasonably temporally robust. The differences occur principally at the longest distances, at which Brewer & Gaston (2003) found several forms of mortality to exhibit positive values of Moran's I which are not shown in the present analyses, but this probably reflects the inclusion of isolated sample sites in central Italy in the previous work, which showed some similarities in holly leaf-miner demographics with those sites sampled in Norway.

Spatial variation in the levels of none of the forms of mortality is correlated with that in leaf-miner density or in measures of host-plant quality. First, this confirms the lack of any simple influence of host-plant

quality on leaf-miner abundance. Secondly, it confirms that none of the forms of leaf-miner mortality are directly driven by host-plant quality.

#### SYNTHESIS

The population dynamics of the holly leaf-miner across Europe are complex. At any site, the mortality that a population experiences is the sum of largely independent yet spatially structured components against a background of varying host-plant quality. Despite lacking any marked spatial structure, host-plant quality may have important local effects. These are difficult to detect regionally, and thus may principally contribute noise to regional patterns of levels of oviposition, abundance and mortality.

#### Acknowledgements

This work was supported by NERC grant GR9/04564/ and The Royal Society. We are grateful to A.M. Brewer, E. Clarke, S.J. Colsell and A. Genney for assistance in the field and the laboratory, and to A.M. Brewer, J. Klok and M.A. McGeoch for discussion. We would also like to thank the many landowners for access to sample sites and P.H. Salvesen and A.E. Haugh for helping us locate Norwegian holly sites.

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Received 1 October 2003; accepted 9 February 2004