

# Geographical variation in host–ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark

THOMAS DAMM ALS,<sup>1</sup> DAVID R. NASH<sup>1,2</sup> and JACOBUS J. BOOMSMA<sup>1,2</sup>

<sup>1</sup>Department of Ecology and Genetics, University of Aarhus and <sup>2</sup>Zoological Institute, Department of Population Ecology, University of Copenhagen, Denmark

**Abstract.** 1. *Maculinea alcon* uses three different species of *Myrmica* host ants along a north–south gradient in Europe. Based on this geographical variation in host ant use, Elmes *et al.* (1994) suggested that *M. alcon* might consist of three or more cryptic species or host races, each using a single and different host-ant species.

2. Population-specific differences in allozyme genotypes of *M. alcon* in Denmark (Gadeberg & Boomsma, 1997) have suggested that genetically differentiated forms may occur in a gradient across Denmark, possibly in relation to the use of different host ants.

3. It was found that two host-ant species are indeed used as hosts in Denmark, but not in a clear-cut north–south gradient. Furthermore, specificity was not complete for many *M. alcon* populations. Of five populations investigated in detail, one used primarily *M. rubra* as a host, another exclusively used *M. ruginodis*, while the other three populations used both ant species. No population in Denmark used *M. scabrinodis* as a host, although this species was present in the habitat and is known to be a host in central and southern Europe.

4. In terms of number of parasites per nest and number of nests parasitised, *M. rubra* seems to be a more suitable host in populations where two host species are used simultaneously. Host-ant species has an influence on caterpillar size but this varies geographically. Analyses of pupae did not, however, show size differences between *M. alcon* raised in *M. rubra* and *M. ruginodis* nests.

5. The geographical mosaic of host specificity and demography of *M. alcon* in Denmark probably reflects the co-evolution of *M. alcon* with two alternative host species. This system therefore provides an interesting opportunity for studying details of the evolution of parasite specificity and the dynamics of host-race formation.

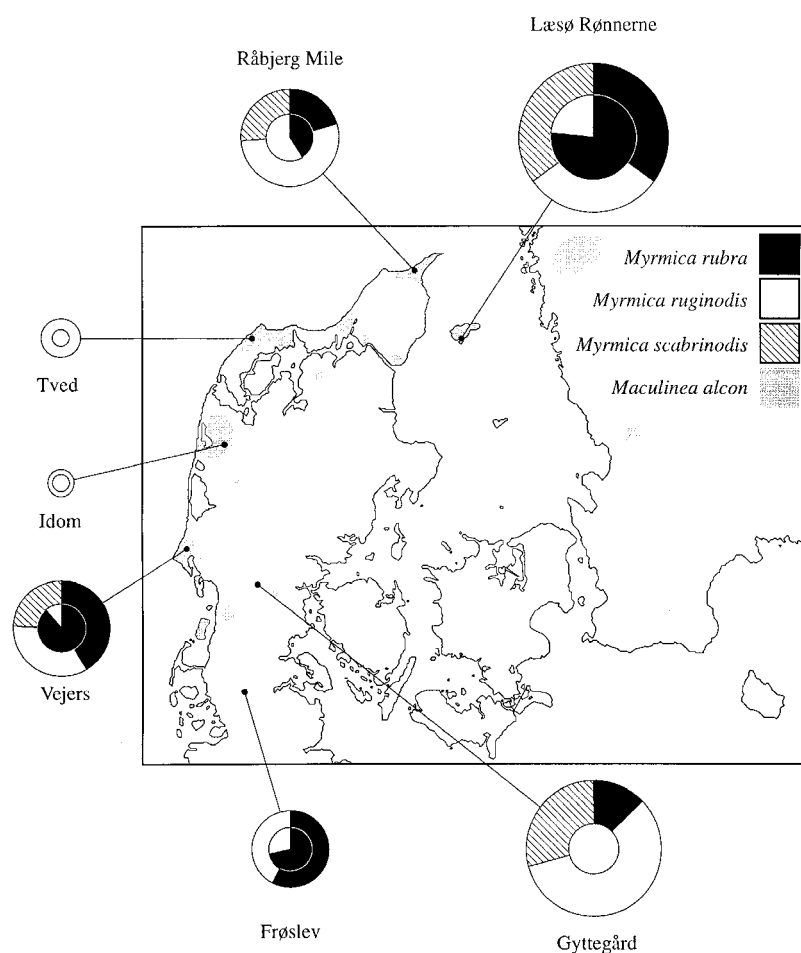
**Key words.** Clumped distribution, geographical variation, host specificity, *Maculinea alcon*, *Myrmica*, social parasitism.

## Introduction

The larvae and pupae of many species in the butterfly family Lycaenidae possess adaptations that enable them to interact with ants (Fiedler, 1991). This association with ants

is mediated by a combination of chemical, nutritional, and physical communication mechanisms (Fiedler *et al.*, 1996; Pierce & Nash, 1999). Lycaenid–ant interactions can be neutral, commensal, competitive, or parasitic but most lycaenid–ant associations are believed to be mutualistic (Pierce, 1987; Fiedler, 1991). Several studies (Atsatt, 1981; Pierce & Mead, 1981; Pierce & Easta, 1986; Pierce *et al.*, 1987; Fiedler & Maschwitz, 1988; Nash, 1989; Thomas & Wardlaw, 1992; Pierce & Nash, 1999) have shown that lycaenid butterflies gain benefits from their interactions

Correspondence: Thomas Damm Als, Department of Ecology and Genetics, University of Aarhus, Building 540, Ny Munkegade, DK-8000 Aarhus C, Denmark. E-mail: thomas.als@biology.au.dk



**Fig. 1.** The populations of *Maculinea alcon* used in the study. Pie charts for each population represent the proportion of *Myrmica* nests of each species found within 2 m of *Gentiana pneumonanthe* plants (outer pie) and the proportion of infested nests of each *Myrmica* species (inner pie). The area of each pie is proportional to the number of ant nests. The distribution of *M. alcon* in Denmark and Sweden is shown as shaded areas, after Stoltze (1996), Wynhoff (1998), and Appelqvist *et al.* (1998).

with ants through their exploitation of ant resources or protective abilities. Costs are also involved, however, as increased utilisation of and intimacy with ants requires greater specialisation, restricting these butterfly species to narrower niches than those of their generalist relatives (Smiley *et al.*, 1988; Nash, 1989; Thomas & Elmes, 1998; Pierce & Nash, 1999). There are two types of specialist lycaenid butterfly: the obligate mutualists and the parasites. The obligate mutualists are restricted to patches where their food plants co-exist with a single genus of ant (Pierce & Elgar, 1985; Pierce, 1987; Smiley *et al.*, 1988; Fiedler, 1994, 1998; Seufert & Fiedler, 1996). The parasitic, aphytophagous species (Cottrell, 1984) in addition need to have highly specialised adaptations for entering ant colonies, which usually also result in high levels of host specificity. Well-known examples in the latter category are species of the genus *Maculinea* van Eecke. The five European species of *Maculinea* exploit one or a few species of the ant genus *Myrmica* Latreille exclusively (Thomas *et al.*, 1989; Elmes *et al.*, 1994), although Japanese *Maculinea* species use

species of the closely-related ant genus *Aphaenogaster* (Yamaguchi, 1988).

The genus *Maculinea* is thought to have evolved in the steppes of central Asia (Sibatani *et al.*, 1994), so that the European *Maculinea* species were pre-adapted to survive and spread in traditional European agricultural landscapes. They are now all threatened with extinction in western Europe, probably through recent changes in land use (Elmes & Thomas, 1992; Thomas, 1995). All species are initially phytophagous, with females ovipositing on the inflorescences of specific perennial host plants that flower only in mid-summer (Thomas *et al.*, 1998a). The young caterpillars develop quickly to the final, fourth instar before leaving their food plants in late July or August. They then apparently mimic the larvae of *Myrmica* ants to ensure that they are found and adopted by foraging *Myrmica* workers (Elmes *et al.*, 1991a; Akino *et al.*, 1999; D. R. Nash, unpublished). The caterpillars live inside the ant nest for at least 10–11 months, gaining some 98% of their final biomass either as predators eating the ants' brood

(Thomas & Wardlaw, 1990, 1992) or in a cuckoo-like manner, being fed actively by the ants (Elmes *et al.*, 1991a,b). The caterpillars pupate in the ant nests and usually eclose as adults the summer after they were adopted. A proportion of the caterpillars of *M. rebeli* Hirschke and *M. alcon* Denis & Schiffermüller takes 2 years to develop before pupation (Thomas *et al.*, 1998b; Schönrogge *et al.*, 2000).

The behaviour and chemical mimicry of newly moulted, last-instar caterpillars ensure that many are discovered and adopted by *Myrmica* workers if their food plant grows within the foraging range of a *Myrmica* nest, typically a distance of some 2 m (Thomas *et al.*, 1989; Elmes *et al.*, 1991a). On *Maculinea* sites in Europe, up to eight species of *Myrmica* may forage beneath the *Maculinea* host plants, and are apparently equally good at finding the caterpillars and carrying them into their nests (Thomas *et al.*, 1989; Elmes *et al.*, 1991a), although there is variation in the speed with which *M. alcon* caterpillars are adopted by different *Myrmica* species in the laboratory (Als *et al.*, 2001).

Earlier work has suggested that in western Europe each *Maculinea* species, once adopted, is raised most successfully to adulthood by a single species of *Myrmica* host ant (Thomas *et al.*, 1989), although secondary hosts sometimes rear a small fraction of the population (Figurny & Woyciechowski, 1998; Thomas & Elmes, 1998). *Maculinea alcon* is a known exception, using different ant species successfully as major hosts in different parts of its western European distribution: *Myrmica scabrinodis* Nylander in the south, *M. ruginodis* Nylander in central Europe, and *M. rubra* (Linnaeus) in the north (Elmes *et al.*, 1994). Elmes *et al.* therefore suggested that *M. alcon* might consist of three subspecies or cryptic species each using a different species of *Myrmica* as host, possibly stemming from the re-invasion of Europe from three allopatric glacial refugia (*cf.* Hewitt, 1999). There is increasing evidence that other *Maculinea* species, particularly *M. rebeli*, may have more than one major host-ant species in western Europe (Jutzeler, 1989; M. Hochberg, pers. comm.). The marsh gentian *Gentiana pneumonanthe* Linnaeus is the main host plant of the first three instars of *M. alcon* throughout most of its western European distribution (Tolman & Lewington, 1997; but see also Marktanner, 1985), regardless of which host ant species is used. The distribution of *M. alcon* and its host plant and host-ant use in the eastern part of its range are less well known (Wynhoff, 1998).

Population-specific differences in allozyme genotypes of *M. alcon* in Denmark (Gadeberg & Boomsma, 1997) suggest that genetically differentiated forms may also occur in this subsection of the species' distribution, with differences between the populations of the north and the south-west. This genetic variation is at least partially consistent with variation in wing pattern (Kaaber, 1964). There are also indications that two gene pools might co-exist in certain populations, possibly in relation to the use of different host ants, because populations are significantly less heterozygous than expected (Gadeberg & Boomsma, 1997). Polish populations of *Maculinea nausithous* Bergsträsser and *M. teleius* Bergsträsser, which both use a single host-ant

species, but otherwise have similarly fragmented populations to *M. alcon*, show no such lack of heterozygosity (Figurny-Puchalska *et al.*, 2000), however detailed knowledge of the actual distribution and exploitation of potential host ants in Danish *M. alcon* populations is lacking. Such analysis is presented here.

## Methods

### Field sites

Seven *Maculinea alcon* populations from Jutland, Denmark (Fig. 1) were investigated from late May to the end of July in 1997, 1998, and 1999. These were a subsample of the populations investigated by Gadeberg and Boomsma (1997). The population closest to the Swedish populations of *M. alcon* (thought to use *M. rubra* exclusively as a host; Elmes *et al.*, 1994) is Læsø Rønnerne (57°14'N, 11°1'E), situated on the island of Læsø, north-east of the Jutland peninsula. The remaining six populations were situated on mainland Jutland. Råbjerg Mile (57°38'N, 10°24'E) in the north, Vejers (55°37'N, 8°12'E) in the west, Gyttegård (55°43'N, 9°3'E) in the mid-west, and Frøslev (54°49'N, 9°17'E) in the south represent some of the largest populations of *M. alcon* in Denmark (Stoltze, 1996; Gadeberg, 1997; Gadeberg & Boomsma, 1997). Populations at Tved (57°5.2'N, 8°38'E) and Idom (56°20'N, 8°28'E) in the north-west were much smaller and were investigated in less detail.

### Checking *Myrmica* nests in early summer

From late May to mid-July, just before the adult butterflies started to emerge from the ant colonies, the seven sites were searched for host plants (*Gentiana pneumonanthe*). When patches of gentians were identified, the area around them was searched for *Myrmica* colonies that occurred within 2 m of gentian plants. At this time of year, the *Myrmica* nests usually have well-developed solaria, and any *M. alcon* larvae present are carried into these or the upper chambers of the nest during the day. The solaria and upper chambers of any *Myrmica* nests found were therefore opened carefully to check for *M. alcon* caterpillars or pupae. Any larvae or pupae found were counted and photographed against a measurement scale, then returned carefully to the ant nest. In 1997, emergence traps were also placed over 40 nests in which no larvae or pupae were found, in order to determine the efficiency of the searching technique. In only one case did a single adult butterfly emerge from a nest that was scored as uninfested. The species of *Myrmica* in each nest was identified using a 10× hand lens in the field, and a sample of five to ten workers was collected and preserved in alcohol for confirmation in the laboratory. Identification keys by Kutter (1977), Collingwood (1979), Seifert (1996), and Wardlaw *et al.* (1998) were used.

Examination of the distribution and density of *Myrmica* colonies, gentian plants, and *M. alcon* eggs at the five main field sites showed that the distribution of *M. alcon* eggs at the end of the flight season was independent of the presence or absence and species of *Myrmica* nests (D. R. Nash, T. D. Als, and J. J. Boomsma, unpublished), which is in accordance with the findings of other studies (van Dyck *et al.*, 2000; Thomas & Elmes, 2001; see discussion). Hence, adoption of *M. alcon* caterpillars by the different *Myrmica* species was assumed to be in proportion to the number of nests of each species within 2 m of gentian plants.

#### Host specificity

For comparison of the strength of host specificity in Danish populations with published data for other European populations, the index *F* (proportion of adopted caterpillars that develops in the primary host nest) developed by Thomas and Elmes (1998) was calculated for the major host ant found on each site:

$$F = \frac{b(1-a)}{a(1-b)}$$

where *a* is the proportion of *Myrmica* ant nests of the primary host species within 2 m of *M. alcon* host plants, and *b* is the proportion of all the fully grown *M. alcon* caterpillars or pupae found within nests of the primary host species at a site. *F* can thus take values between 1 (no specificity; caterpillar distribution mirrors that of the potential host ants) and  $\infty$  (complete specificity, all caterpillars are found in the nests of one host species).

For each site, the relative success of *M. alcon* in *M. rubra* and *M. ruginodis* nests was tested using a Fisher's exact test (Sokal & Rohlf, 1995), the null hypothesis being that the proportion of nests of each ant species that reared *M. alcon* caterpillars successfully through the winter is expected to equal the proportion of nests of each species within 2 m of gentian plants at each site.

The frequency distribution of the number of *M. alcon* reared in the nests of *M. rubra* and *M. ruginodis* was highly overdispersed. Negative binomial distributions were therefore fitted to these data, following the methods outlined by Krebs (1989) for the estimation of distribution parameters and testing goodness of fit. Differences in the number of *M. alcon* caterpillars reared in parasitised nests of *M. rubra* and *M. ruginodis* were tested using a Wilcoxon–Mann–Whitney test (Zar, 1996) for all sites where both hosts were used. Analyses were carried out using SPSS 8.0 for PC (Norusis, 1999), JMP 3.2.2 for Macintosh (SAS Institute Inc., 1995), GLMStat 5.6.1 for Macintosh (Beath, 2001), or by hand using Excel spreadsheets.

#### Measuring caterpillars and pupae

Caterpillar and pupa measurements were made from photographic slides taken in the field. The length and width were measured for each caterpillar and pupa, and in

addition, wing length was measured for each pupa. The length of the measurement scale photographed together with the caterpillars and pupae was then used to calibrate these measurements. For the analysis, the volume *V* of each caterpillar or pupa was estimated, calculated as a cylinder with hemispherical ends using the formula:

$$V = \pi r^2(l - 2r) + \frac{4\pi r^3}{3}$$

where *r* is the radius of the cylinder (and its hemispherical ends) and *l* is the total length of the caterpillar or pupa. While this method may be more subject to measurement error than, for example, weighing caterpillars and pupae in the field (e.g. Thomas *et al.*, 1998b), it was chosen so as to cause as little disturbance as possible to the caterpillars and pupae, and to allow them to be returned to their host nests as quickly as possible. The measurement error in caterpillar size is likely to be greater than that for pupal size but should be equally distributed across sites and ant species.

The median pupation date of the *M. alcon* at each site was estimated and compared by fitting a general linear model with binomial errors to the data on caterpillar and pupal numbers from each site (Crawley, 1993). The number of pupae found in each nest was used as the response variable, and the sum of the number of caterpillars and pupae in each nest was used as the binomial denominator. Site, year, host-ant species, and day of the year (number of days since 1 January) were fitted as explanatory variables.

Mean caterpillar volume and mean pupa volume were calculated for each host-ant nest and used in the subsequent analyses. For caterpillar volume, only those caterpillars expected to pupate in the current year were used (see results). Not all populations were sampled in all 3 years, and not all populations provided data for both ant species, leading to a highly unbalanced design. The effects of site, ant species, and year on caterpillar and pupae volumes were therefore analysed using a general linear model with normal errors. Only nests from the five sites examined in detail were used in this analysis. The unbalanced design meant that the three-way interaction among year, site, and host species could not be fitted as part of the model. Significance testing was performed using stepwise elimination of terms from the maximal model, which is the most suitable procedure for examination of the effects of non-experimental factors (Crawley, 1993).

## Results

#### Distribution of *M. alcon* in host nests

A total of 516 *Myrmica* colonies within foraging range of *G. pneumonanthe* plants was investigated at the seven sites. The proportions of the different *Myrmica* species found are given in Table 1 and shown graphically in Fig. 1. Only *M. rubra*, *M. ruginodis*, and *M. scabrinodis* were found within 2 m of gentian plants at the investigated sites; these are the three species recorded as hosts of *Maculinea alcon*

**Table 1.** The number of *Myrmica* nests within 2 m of a *Gentiana pneumonanthe* plant examined at each of seven sites, and the number with caterpillars or pupae of *M. alcon* present. The statistical significance of the differences in the proportion of parasitised nests of *M. rubra* and *M. ruginodis* at each site is based on a two-tailed Fisher's exact test. The index of specificity *F* is calculated following Thomas and Elmes (1998); see text for details.

Site	Ant species	Sample size	Number with <i>M. alcon</i>	<i>P</i>	Total number of <i>M. alcon</i>	Specificity ( <i>F</i> )
Læsø Rønnerne	<i>M. rubra</i>	60	43	< 0.001	391	5.02
	<i>M. ruginodis</i>	51	13		144	
	<i>M. scabrinodis</i>	60	0		0	
Råbjerg Mile	<i>M. rubra</i>	15	7	0.208	100	8.74
	<i>M. ruginodis</i>	40	11		45	
	<i>M. scabrinodis</i>	19	0		0	
Tved	<i>M. ruginodis</i>	11	2	—	10	—
Idom	<i>M. ruginodis</i>	5	2	—	13	—
Vejers	<i>M. rubra</i>	29	16	< 0.001	86	7.15
	<i>M. ruginodis</i>	24	2		17	
	<i>M. scabrinodis</i>	17	0		0	
Gyttegård	<i>M. rubra</i>	18	0	0.020	0	∞
	<i>M. ruginodis</i>	81	19		67	
	<i>M. scabrinodis</i>	41	0		0	
Frøslev	<i>M. rubra</i>	26	10	0.330	31	2.83
	<i>M. ruginodis</i>	19	4		8	

somewhere in its range (Elmes *et al.*, 1994). Other *Myrmica* species (e.g. *M. sulcinodis*) were found occasionally at a greater distance from gentian plants. *Myrmica ruginodis* was present on all seven sites. *Myrmica rubra* and *M. scabrinodis* were absent at Tved and Idom, where the area of suitable habitat was very small (Gadeberg, 1997; Gadeberg & Boomsma, 1997), and *M. scabrinodis* was absent from Frøslev.

In Tved and Idom, all *M. alcon* individuals were found in nests of *M. ruginodis*, as this was the only *Myrmica* species present at those sites. In Gyttegård, all three ant species were present within foraging range of host plants but *M. alcon* caterpillars and pupae were only found in nests of *M. ruginodis*. In Vejers, all three ant species were present but only *M. rubra* was a host in 1997 and 1998, although two parasitised nests of *M. ruginodis* were found in 1999. At Råbjerg Mile and Læsø Rønnerne, all three ant species were present and both *M. rubra* and *M. ruginodis* were parasitised in all years. In Frøslev, only *M. rubra* and *M. ruginodis* were present within foraging range of host plants, and both species were parasitised in all years.

In all populations where both *M. rubra* and *M. ruginodis* were used as hosts, a greater proportion of available *M. rubra* nests was parasitised successfully by *M. alcon* (Table 1). This difference was significant for the large samples obtained from Læsø and Vejers but not for the somewhat smaller samples from Råbjerg Mile and Frøslev. The values of the host specificity index *F* ranged from 2.83 to 8.74 for the populations where *M. rubra* was the major host. The population of *M. alcon* at Gyttegård was only found in nests of *M. ruginodis*, resulting in a value of the host specificity index *F* of ∞.

The distributions of the number of *Maculinea* caterpillars, pupae, and adults found in the nests of *Myrmica rubra* and *M. ruginodis* are given in Fig. 2. In general, distributions were highly clumped and can be described by negative binomial distributions with dispersion parameter  $k < 1$

(Table 2). The dispersion parameter  $k$  was significantly lower for *M. alcon* in nests of *M. ruginodis* (mean  $\pm$  SE =  $0.14 \pm 0.03$ ) than for *M. alcon* in nests of *M. rubra* ( $0.34 \pm 0.05$ ; Wilcoxon–Mann–Whitney test:  $m = 4$ ,  $n = 7$ ,  $W_x = 36$ ,  $P < 0.05$ ), indicating a more clumped distribution of *M. alcon* in *M. rubra* nests than in *M. ruginodis* nests.

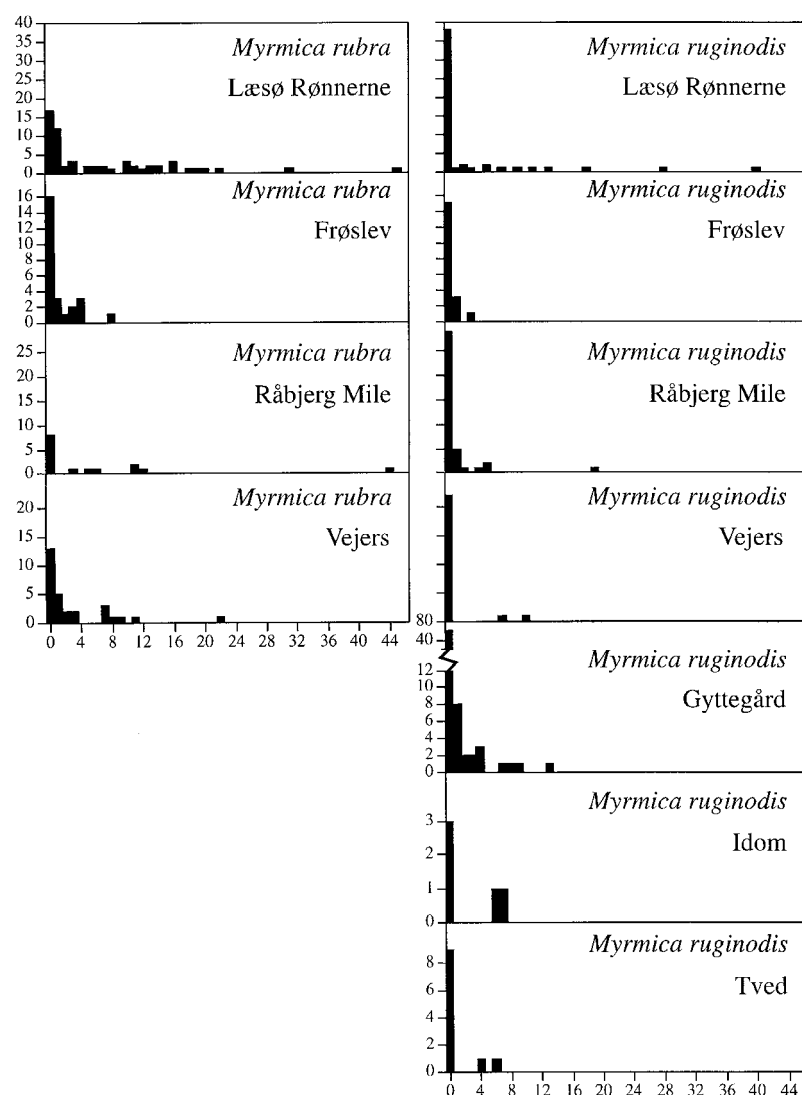
Comparison of the number of *M. alcon* caterpillars and pupae found in infested *M. rubra* and *M. ruginodis* nests showed no significant difference for three of the sites where both hosts were used (Læsø Rønnerne, Vejers, and Frøslev), but a significantly greater number of *M. alcon* larvae was found in *M. rubra* nests at Råbjerg Mile (Table 2).

#### Median pupation dates of *M. alcon*

The minimal adequate model comparing the proportion of juvenile *M. alcon* that had pupated contained only day of the year and the interaction between day of the year and site (general linear model, change in scale deviance = 16.54, d.f. = 4,  $P < 0.01$ ). This shows that median pupation date differed significantly among sites but not among years or different host-ant species. The median pupation date for each site together with its 95% confidence intervals, calculated using the method outlined by Collett (1991), are shown in Fig. 3. It is clear that *M. alcon* larvae on the island of Læsø pupate earlier than *M. alcon* larvae at other sites.

#### Caterpillar and pupal sizes

A total of 430 caterpillars and 422 pupae was measured. Schönrogge *et al.* (2000) showed that there is a polymorphism in the development of *M. alcon*, with a proportion of larvae taking 2 years to develop. There is also evidence of a



**Fig. 2.** Frequency distributions of the number of caterpillars per colony for the two host species *Myrmica rubra* and *M. ruginodis* at the seven sites.

bimodal distribution of caterpillar sizes, with the smallest caterpillars not pupating in the current year. The data for frequency distribution of caterpillar and pupal volumes were split into three groups: caterpillars and pupae collected >5 days before the median pupation date for each site, those collected between 5 days before and 12 days after the median pupation date, and those collected more than 12 days after the median pupation date (Fig. 4). These time intervals were chosen to divide the period over which data were collected into three similarly sized samples. During periods 1 and 2, there was a bimodal distribution of caterpillar sizes, with one peak occurring below a caterpillar size of 40 mm<sup>3</sup> and a second, broader peak above this size. For period 3, there was a unimodal distribution of caterpillar sizes, which hardly overlapped with the pupal size distribution. It seems reasonable to assume that the lower distributional peak in periods 1

and 2 represents those small caterpillars that will not pupate in the year of sampling, and that no caterpillars in period 3 will pupate in the year of sampling. These caterpillars were therefore excluded from the analysis of caterpillar size.

The results of the analysis of caterpillar and pupal sizes are given in Table 3, with mean volumes of caterpillars and pupae shown in Fig. 5. There was a significant site  $\times$  species interaction for the analysis of caterpillar sizes, showing that host-ant species had an effect on caterpillar size, but the magnitude and direction of this effect varied from site to site. Caterpillars reared in *M. rubra* nests were smaller than those reared in *M. ruginodis* nests at Råbjerg Mile and Vejers, but similar in size at Læsø Rønnerne (Fig. 5). The analysis of pupal volumes showed no significant differences in pupal volume among years, species, or any interactions, but an effect of site that was close to significance.

**Table 2.** The distribution of individuals of *M. alcon* found in *Myrmica* nests at the seven field sites.  $k$  is the negative binomial clumping parameter.  $P$ -values refer to Wilcoxon–Mann–Whitney tests investigating the difference in numbers of *M. alcon* developing in each infested nest of *M. rubra* and *M. ruginodis*. Because four independent tests were made, Bonferroni correction was necessary, and  $p$ -values should be regarded as significant if less than 0.0125. See text for details.

Site	Ant species	Number of <i>M. alcon</i> per ant nest			Number of <i>M. alcon</i> per infested ant nest		
		Mean	SD	$k$	Mean	SD	$P$
Læsø Rønnerne	<i>M. rubra</i>	6.52	8.79	0.44	9.09	9.20	0.571
	<i>M. ruginodis</i>	2.84	7.51	0.09	11.08	11.55	
Råbjerg Mile	<i>M. rubra</i>	6.67	11.55	0.21	14.29	13.59	0.009
	<i>M. ruginodis</i>	1.13	3.24	0.17	4.09	5.26	
Tved	<i>M. ruginodis</i>	0.91	2.07	0.09	5.00	1.41	–
Idom	<i>M. ruginodis</i>	2.60	3.58	0.25	6.50	0.71	–
Vejers	<i>M. rubra</i>	2.97	4.90	0.36	5.38	5.57	0.225
	<i>M. ruginodis</i>	0.71	2.44	0.03	8.50	2.12	
Gyttegård	<i>M. ruginodis</i>	0.83	2.21	0.14	3.53	3.41	–
Frøslev	<i>M. rubra</i>	1.19	2.00	0.34	3.10	2.13	0.303
	<i>M. ruginodis</i>	0.42	0.96	0.24	2.00	1.15	

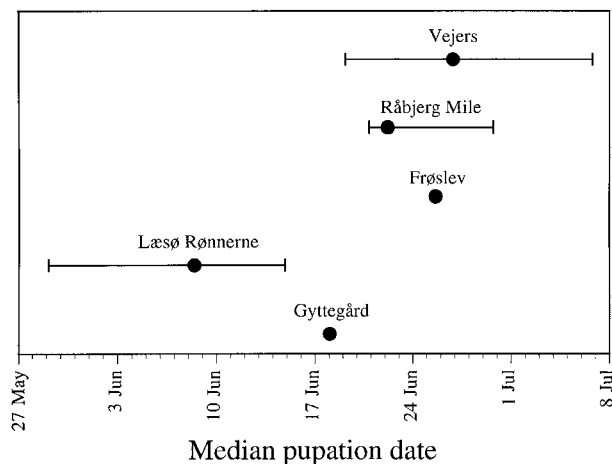
## Discussion

### Distribution among populations

The current study confirms that two ant species, *M. rubra* and *M. ruginodis*, are used as hosts by *Maculinea alcon* in Denmark, but not in a clear-cut cline as predicted by Gadeberg and Boomsma (1997). When the pattern of host use from the populations used in this study is compared with the allozyme data for the same populations published by Gadeberg and Boomsma (1997), it is clear that the populations with the same pattern of host use are not

those that are most similar genetically, at least based on the allozyme markers used by Gadeberg and Boomsma. This suggests that the south-west to north-east cline in allozyme frequencies found by these authors is not the result of a cline in host use but must have another explanation.

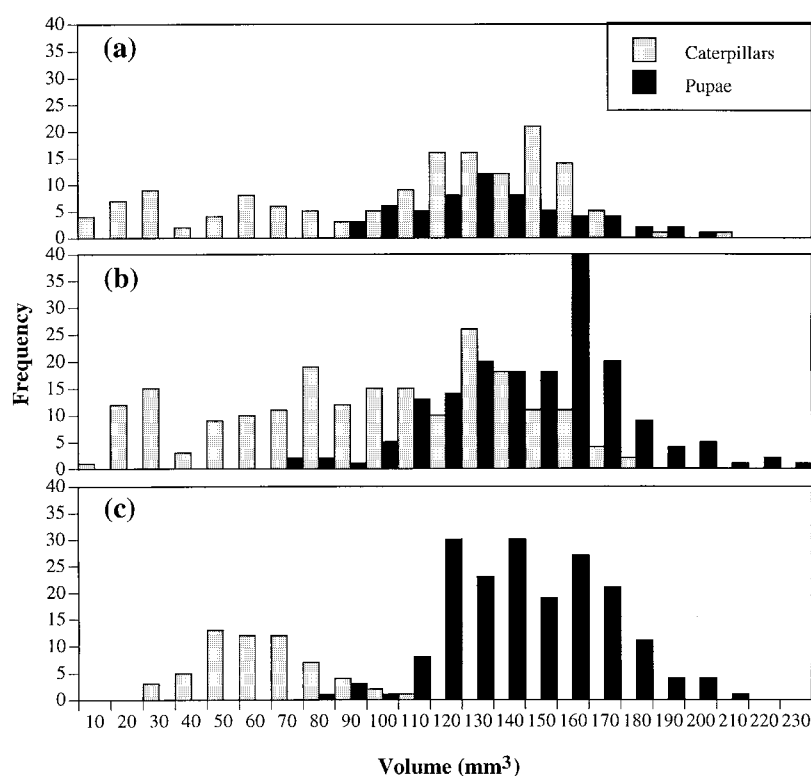
Host specificity is, however, not complete for several *M. alcon* populations: of the five populations investigated in detail, one, Gyttegård, used exclusively *M. ruginodis*, but the other four used both *M. rubra* and *M. ruginodis* within the same site. None of the populations parasitised *M. scabrinodis*, although it was present at four of the five sites investigated most thoroughly. The finding that two host species are used simultaneously at several sites could reflect, at one extreme, low specificity of *M. alcon*, or at the other, the existence of two genetically distinct sub-populations with complete host specificity. With the current data, it is not possible to say where on the continuum of possibilities between these two extremes the populations of *M. alcon* in Denmark lie. Gadeberg and Boomsma (1997) found an excess of homozygote genotypes (high positive  $F_{IS}$  values) for populations of *M. alcon* in Denmark, which could be consistent with several co-existing but partially reproductively-isolated *M. alcon* gene pools, but the absence of diagnostic loci or alleles prevented them from documenting such a Wahlund effect in the *M. alcon* populations across Jutland (Wahlund, 1928). More powerful DNA techniques and crossing experiments between butterflies reared by different host-ant species will be needed to confirm or reject the existence of host races or cryptic species of *M. alcon*.



**Fig. 3.** The median date of pupation, calculated on the basis of records of both larvae and pupae for each site from a variety of dates. Bars indicate 95% confidence intervals calculated following Collett (1991). For two sites (Frøslev and Gyttegård), confidence intervals could not be calculated because sample sizes were too low. Sites are arranged according to host-ant use, i.e. Vejers using primarily *M. rubra*, Råbjerg Mile, Frøslev, and Læsø Rønnerne using both *M. rubra* and *M. ruginodis*, Gyttegård using only *M. ruginodis*.

### Distribution within populations

Earlier studies (Thomas, 1984; Thomas *et al.*, 1989; Elmes *et al.*, 1991a, 1996; Thomas & Elmes, 1998) were not able to detect differences in the survival of eggs or caterpillars on the initial food plants growing in the slightly different



**Fig. 4.** Frequency distribution of the sizes of caterpillars and pupae measured as estimated volume (see text). (a) Samples taken more than 5 days before the median pupation date, (b) samples taken between 5 days before and 12 days after the median pupation date, and (c) samples taken >12 days after the median pupation date.

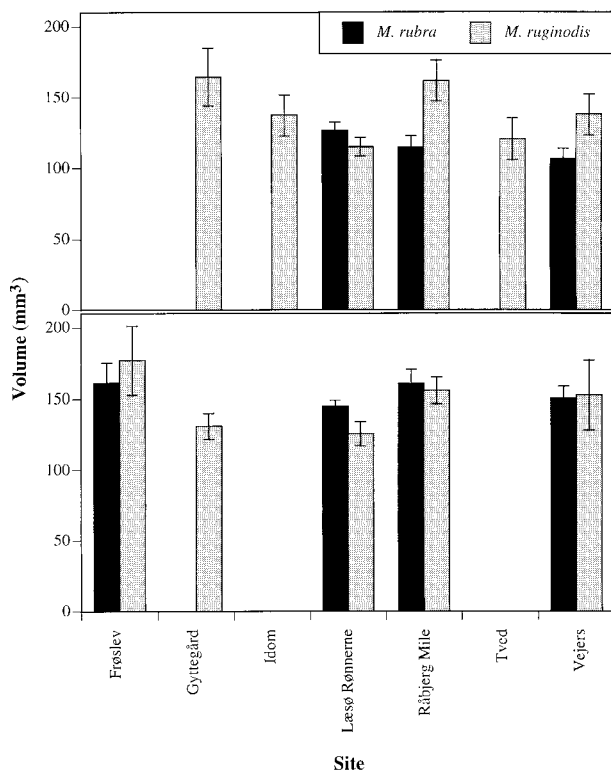
microhabitats preferred by different *Myrmica* species for any of the five European species of *Maculinea*. Likewise they were not able to detect biases in the initial adoption of young final-instar *Maculinea* caterpillars into the nests of particular species of *Myrmica*. Pierce and Elgar (1985) cited observations by Frohawk (1903), which indicated that *M. arion* Linnaeus females oviposit in the vicinity of *Myrmica* ant nests, but subsequent studies of *M. arion*,

*M. teleius*, and *M. nausithous* (Thomas, 1984; Elmes & Thomas, 1987) could not confirm ant-dependent oviposition behaviour. Recent work by van Dyck *et al.* (2000) on *M. alcon* in Belgium and the Netherlands has shown that at the start of the flight period there is a significant association between the presence of *M. ruginodis* (presumed to be the host-ant species there) and the gentians that are used by *M. alcon* for oviposition, which these authors interpret as evidence for ant-mediated oviposition. As the flight period progresses, however, oviposition is directed mostly towards gentians without competing *M. alcon* larvae, resulting in a final distribution of *M. alcon* that is unrelated to the distribution of the host ant at one site, and shows a much lower association at a second site. Thomas and Elmes (2001) suggested that the patterns found by van Dyck *et al.* (2000) may be explained better by phenological differences in the host plants due to microhabitat differences, which also reflect ant distribution. Whatever the oviposition cues used by female *M. alcon*, both studies have shown that the number of eggs laid by *Maculinea* females on food plants close to host-ant nests is not significantly different from the number laid close to the nests of other *Myrmica* species. For the sites used for this study, there is also no relationship between the distribution of eggs of *M. alcon* at the end of the flight season and the distribution of the different *Myrmica* ant species (D. R. Nash and T. D. Als, unpublished). Thus, the assumption that the proportion of

**Table 3.** Analysis of the differences in caterpillar and pupal size (measured as volume) among different populations of *M. alcon* (site), different years (year), and different host-ant species (species). Analysis was carried out using a general linear model with normal errors. Only data for the five populations studied in detail (Frøslev, Gyttegård, Læsø Rønnerne, Råbjerg Mile, and Vejers) were included in the analysis.

	Caterpillar volume			Pupal volume		
	d.f.	F	P	d.f.	F	P
Site	3	2.058	0.125	4	2.404	0.059
Species	1	0.881	0.355	1	2.281	0.136
Year	1	0.065	0.801	2	0.137	0.872
Site × species	2	6.888	0.003	3	0.806	0.495
Site × year	1	0.250	0.621	2	0.078	0.925
Year × species	1	0.013	0.910	1	2.003	0.162
Residual	33			62		





**Fig. 5.** Mean caterpillar (upper graph) and pupal (lower graph) sizes, measured as estimated volume (see text) for *M. alcon* raised in *M. rubra* and *M. ruginodis* nests. Error bars represent  $\pm 1$  SE of the mean. Only caterpillars expected to pupate are included (see text).

nests of the different *Myrmica* ant species within 2 m of gentian plants reflects the proportion of nests that will be susceptible to parasitism seems justified.

The proportion of host *Myrmica* nests within 2 m of gentian plants that was parasitised was much higher in this study than the values reported by Thomas and Elmes (1998), who found that 8–10% of host nests in Spain and the Netherlands were infected by *M. alcon*, although the data for the Swedish population they examined (Elmes *et al.*, 1994) showed a parasitism rate of *M. rubra* of 67%. For sites where *M. rubra* was used as the primary host in the present study, parasitism rates of this species ranged from 38 to 72%, and showed significant between-site variation ( $\chi^2 = 9.58$ , d.f. = 3,  $P < 0.05$ ). Parasitism rates of *M. ruginodis* at all sites were 8–40%. Interestingly, the rate of parasitism of *M. ruginodis* nests was relatively constant across sites ( $\chi^2 = 4.57$ , d.f. = 6,  $P = \text{NS}$ ), and independent of whether *M. rubra* or *M. ruginodis* was the primary host at a site (G test for heterogeneity:  $G_{(\text{corrected, Yates})} = 5.83$ , d.f. = 6,  $P = \text{NS}$ ).

At the four sites with two host species, *M. rubra* was the primary host, with the proportion of *M. alcon* developing in *M. ruginodis* nests ranging from 16 to 30%. The host specificity index,  $F$ , used by Thomas and Elmes (1998) is much lower (2.83–8.74) for the populations in this study using *M. rubra* as a primary host than the values reported for

*M. alcon* by Thomas and Elmes (23.5 to  $\infty$ ), and closer to their value for the predacious *M. arion* (6.5). Hence, the conclusion of Thomas and Elmes (1998) that the cuckoo-like parasitism of *M. alcon* leads to greater host specificity does not seem to be borne out in Danish populations. This may, however, be a specific consequence of sampling in the transition zone between populations that use only *M. rubra* as host and populations that use only *M. ruginodis*. The difference in the specificity index arises from a higher proportion of *M. alcon* developing in nests of a secondary host [ $b$  ranges from 0.70 to 0.84 in this study, compared with 0.95–1.0 in Thomas and Elmes (1998);  $t = 3.36$ , d.f. = 4,  $P < 0.05$ ], rather than the primary host making up a smaller proportion of the *Myrmica* nests available to larvae [ $a$  is 0.21–0.58 in this study, 0.46–0.74 in Thomas and Elmes (1998),  $t = 1.48$ , d.f. = 4,  $P = 0.21$ ].

Within the populations where two host species are used simultaneously, the number of larvae and pupae reared per nest was higher in *M. rubra* nests, but only significantly so at Råbjerg Mile. This is consistent with laboratory experiments showing higher post-adoption survival of *M. alcon* larvae in nests of *M. rubra* compared with nests of *M. ruginodis* (D. R. Nash and T. D. Als, unpublished). The cuckoo feeding lifestyle of *M. alcon* means that the numbers of caterpillars that can be raised by each host-ant colony is not limited as directly by the colony size as for predacious species such as *M. arion* (Thomas & Elmes, 1998). Colony size may still, however, set an upper limit to the distribution of numbers of caterpillars found in *Myrmica* nests in the field. Elmes *et al.* (1991b) estimated that approximately 50 workers were required to raise each caterpillar of a cuckoo feeding *Maculinea* species, and models of *M. rebeli* population dynamics based on the assumption of lower survivorship in smaller host nests show a remarkably good fit to empirical data (Elmes *et al.*, 1996). *Myrmica rubra* normally has the largest colony sizes in the genus *Myrmica* in Europe, although *M. ruginodis* colonies may grow exceptionally large under some circumstances (Wardlaw & Elmes, 1996). Higher numbers of caterpillars in the nests of *M. rubra* may therefore simply reflect the larger amount of resources available in larger *M. rubra* colonies. The size of colonies of *M. rubra* was not estimated in the field because this would have been too time-consuming and destructive. *Myrmica rubra* is also highly polydomous, which makes estimation of colony size particularly difficult (Walin *et al.*, 2001). Comparing the data on the numbers of caterpillars of *M. alcon* in host nests with the data presented by Thomas and Elmes (1998) for *M. alcon* and the closely related *M. rebeli* shows that there are generally higher numbers of caterpillars per *Myrmica* nest (this study: mean = 2.5, range 0–44; data from Thomas and Elmes, 1998: for *M. alcon*: mean = 0.47, range 0–20; for *M. rebeli*: mean = 1.21, range 0–22).

The finding that the distribution of *M. alcon* numbers within ant nests fits negative binomial distributions with dispersion parameter  $k < 1$  is typical for macro-parasites (Anderson & May, 1978). The somewhat greater clumping of *M. alcon* larvae in *M. ruginodis* nests suggests that this

host is relatively more variable in its interactions with *M. alcon* than is *M. rubra*, with some colonies able to avoid parasitism better than others. This is again consistent with laboratory experiments, in which adoption time of *M. alcon* caterpillars by *M. ruginodis*, and their subsequent survival is more variable in *M. ruginodis* than in *M. rubra* colonies (Als *et al.*, 2001; D. R. Nash *et al.*, unpublished).

#### Development of *M. alcon*

Pupation took place earlier on the Island of Læsø than in the other populations of *M. alcon*. This is consistent with records of flying adults, which are usually seen on Læsø before they are seen on mainland Jutland. Data on labels in collections of *M. alcon* adults in the Natural History Museum in Aarhus support this; *M. alcon* flies significantly earlier in the north-east (mean flight day 14 July, range 30 June to 5 August; most of the data are from Læsø) than in the rest of Jutland (mean flight day 19 July, range 4 July to 13 August). The sizes of caterpillars of *M. alcon* in the field early in the period during which pupation takes place show a characteristic bimodal distribution, as also observed by Elfferich (1963). Later in the season, only pupae and small caterpillars are found in *Myrmica* nests. Both these observations are consistent with a fraction of *M. alcon* taking 2 years to develop (Thomas *et al.*, 1998b; Schönrogge *et al.*, 2000).

Analyses of the sizes of caterpillars that are likely to pupate in the study year showed that caterpillars reared by different host species were of different sizes, but that the magnitude and direction of these differences varied from population to population. This result is also consistent with laboratory experiments, in which caterpillars originating from different sites have been found to have somewhat different growth trajectories in the nests of different host-ant species (D. R. Nash and T. D. Als, unpublished). Differences in caterpillar size disappeared once the caterpillars had pupated. Because fecundity of insects, and particularly butterflies, is correlated with adult, and hence pupal, size (e.g. Elgar & Pierce, 1988), this suggests that there is unlikely to be an overall difference in reproductive success between *M. alcon* reared by *M. rubra* and *M. ruginodis*.

#### Conclusion

The results of this study show that host specificity and phenology of *M. alcon* in Denmark vary spatially but not in a simple north-south or east-west cline. Populations of *M. alcon* may represent a geographical mosaic as a result of the co-evolution between the butterfly and its alternative host-ant species (Thompson, 1999). *Maculinea alcon* is an endangered or threatened species in many parts of Europe (Elmes *et al.*, 1994), and declined considerably in Denmark during the 20th Century (Stoltze, 1996). The geographical variation in the interactions of this species with its *Myrmica* ant hosts shown in Denmark has important consequences for its conservation, resulting in

demographic fragmentation that is not obvious from standard distribution maps.

#### Acknowledgements

We are grateful to Rebekka Gadeberg for advice and invaluable information on Danish *Maculinea alcon* sites, Jes Søb Pedersen, Graham Elmes and Jeremy Thomas for valuable suggestions and comments, Nordjyllands Statskovdistrikt for allowing field work to take place on Læsø Rønnerne and Råbjerg Mile, Steffen Jørgensen (Randbøl skovdistrikt) for allowing the field survey to take place at Gyttegård, Dr Jur. Beckmann for allowing fieldwork at Frøslev and Mark Desholm and Anne Birgitte Als for field assistance. An anonymous referee made useful comments on the manuscript. D.R.N. was supported by a EU Marie Curie fellowship (ERBFMBICT 972161) and by an individual fellowship from the Carlsberg foundation.

#### References

- Akino, T., Knapp, J.J., Thomas, J.A. & Elmes, G.W. (1999) Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proceedings of the Royal Society of London, Series B*, **266**, 1419–1426.
- Als, T.D., Nash, D.R. & Boomsma, J.J. (2001) Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae) by three *Myrmica* ant species. *Animal Behaviour*, **62**, 99–106.
- Anderson, R.M. & May, R.M. (1978) Regulation and stability of host-parasite population interactions. *Journal of Animal Ecology*, **47**, 219–247.
- Appelqvist, T., Grimdal, R., Finsberg, M. & Bengtson, O. (1998) Alkonblåvingen (*Maculinea alcon*) i Västra Götalands län – habitatval och naturvårdsaspekter. *Entomologisk Tidskrift*, **119**, 121–130.
- Atsatt, P.R. (1981) Lycaenid butterflies and ants: selection for enemy-free space. *American Naturalist*, **118**, 638–654.
- Beath, K.J. (2001) *GLMStat: Generalised Linear Modelling Software*. <http://members.ozemail.com.au/~kjbeath/glmstat.html>
- Collett, D. (1991) *Modelling Binary Data*. Chapman & Hall, London.
- Collingwood, C.A. (1979) *The Formicidae (Hymenoptera) of Fennoscandia and Denmark – Fauna Entomologica Scandinavica, Vol. 8*. Scandinavian Science Press Ltd, Klampenborg, Denmark.
- Cottrell, C.B. (1984) Aphytophagy in butterflies: its relationship to myrmecophily. *Zoological Journal of the Linnean Society*, **79**, 1–57.
- Crawley, M.J. (1993) *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford.
- van Dyck, H., Oostermeijer, J.G.B., Talloen, W., Wynhoff, I., Feenstra, V. & van der Hidde, A. (2000) Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? *Proceedings of the Royal Society of London, Series B*, **267**, 861–866.
- Elfferich, N.W. (1963) Kweekervaringen met *Maculinea alcon* Schiff. *Entomologische Berichten*, **23**, 46–52.
- Elgar, M.A. & Pierce, N.E. (1988) Mating success and fecundity in an ant-tended lycaenid butterfly. *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems* (ed. by T. H. Clutton-Brock), pp. 59–75. University of Chicago Press, Chicago, Illinois.

- Elmes, G.W., Clarke, R.T., Thomas, J.A. & Hochberg, M.E. (1996) Empirical tests of specific predictions made from a spatial model of the population dynamics of *Maculinea rebeli*, a parasitic butterfly of red ant colonies. *Acta Oecologica – International Journal of Ecology*, **17**, 61–80.
- Elmes, G.W. & Thomas, J.A. (1987) Die Biologie und Ökologie der Ameisen der Gattung *Myrmica*. *Tagfalter und ihre Lebensräume: Arten, Gefährdung, Schutz* (ed. by W. Geiger), pp. 404–409. Schweizerischer Bund für Naturschutz, Basle, Switzerland.
- Elmes, G.W. & Thomas, J.A. (1992) Complexity of species conservation in managed habitats: interaction between *Maculinea* butterflies and their ant hosts. *Biodiversity and Conservation*, **1**, 155–169.
- Elmes, G.W., Thomas, J.A., Hammarstedt, O., Munguira, M.L., Martin, J. & van der Made, J.G. (1994) Differences in host-ant specificity between Spanish, Dutch and Swedish populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.) (Lepidoptera). *Memorabilia Zoologica*, **48**, 55–68.
- Elmes, G.W., Thomas, J.A. & Wardlaw, J.C. (1991a) Larvae of *Maculinea rebeli*, a large-blue butterfly, and their *Myrmica* host ants: wild adoption and behaviour in ant-nests. *Journal of Zoology*, **223**, 447–460.
- Elmes, G.W., Wardlaw, J.C. & Thomas, J.A. (1991b) Larvae of *Maculinea rebeli*, a large-blue butterfly, and their *Myrmica* host ants: patterns of caterpillar growth and survival. *Journal of Zoology*, **224**, 79–92.
- Fiedler, K. (1991) *Systematic, Evolutionary, and Ecological Implications of Myrmecophily Within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea)*. Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany.
- Fiedler, K. (1994) Lycaenid butterflies and plants: is myrmecophily associated with amplified hostplant diversity. *Ecological Entomology*, **19**, 79–82.
- Fiedler, K. (1998) Lycaenid–ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. *Journal of Insect Conservation*, **2**, 3–14.
- Fiedler, K., Hölldobler, B. & Seufert, P. (1996) Butterflies and ants – the communicative domain. *Experientia*, **52**, 14–24.
- Fiedler, K. & Maschwitz, U. (1988) Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants – a quantitative approach. *Oecologia*, **75**, 204–206.
- Figurny, E. & Woyciechowski, M. (1998) Flowerhead selection for oviposition by females of the sympatric butterfly species *Maculinea teleius* and *M. nausithous* (Lepidoptera: Lycaenidae). *Entomologia Generalis*, **23**, 215–222.
- Figurny-Puchalska, E., Gadeberg, R.M.E. & Boomsma, J.J. (2000) Comparison of the genetic population structure of the large blue butterflies *Maculinea nausithous* and *M. teleius*. *Biodiversity and Conservation*, **9**, 419–432.
- Frohawke, F.W. (1903) The earlier stages of *Lycaena arion*. *Entomologist*, **36**, 57–60.
- Gadeberg, R.M.E. (1997) *Populationsgenetiske og økologiske studier af ensianblåfuglen Maculinea alcon i sit udbredelsesområde i Jylland*. Unpublished MSc thesis, University of Aarhus, Denmark.
- Gadeberg, R.M.E. & Boomsma, J.J. (1997) Genetic population structure of the large blue butterfly *Maculinea alcon* in Denmark. *Journal of Insect Conservation*, **1**, 99–111.
- Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.
- Jutzeler, D. (1989) *Maculinea rebeli* (Hirschke): ein Raupenfund im Glarnerland (Lepidoptera: Lycaenidae). *Mitteilung der Entomologische Gesellschaft Basel*, **39**, 92–93.
- Kaaber, S. (1964) Studies on *Maculinea alcon* (Schiff.)-*rebeli* (Hir.) (Lep. Lycaenidae) with reference to the taxonomy, distribution, and phylogeny of the group. *Entomologiske Meddelelser*, **32**, 277–319.
- Krebs, C.J. (1989) *Ecological Methodology*. Harper Collins, New York.
- Kutter, H. (1977) *Hymenoptera, Formicidae – Fauna Insecta Helvetica*, 6. Schweizerische Entomologische Gesellschaft, Zurich, Switzerland.
- Marktanner, T. (1985) Die Bedeutung des Schwalbenwurzengians als Futterpflanze von *Maculinea alcon* (Lep. Lycaenidae) im oberschwäbischen Alpenvorland und die Verbreitung der Lycaenide in diesem Raum. *Entomologische Zeitschrift*, **95**, 257–272.
- Nash, D.R. (1989) *Cost-benefit analysis of a mutualism between lycaenid butterflies and ants*. Unpublished D. Phil. thesis, University of Oxford.
- Norusis, M.J. (1999) *SPSS 8.0 Guide to Data Analysis*. Prentice Hall, Upper Saddle River, New Jersey.
- Pierce, N.E. (1987) The evolution and biogeography of associations between lycaenid butterflies and ants. *Oxford Surveys in Evolutionary Biology* (ed. by P. H. Harvey and L. Partridge), pp. 89–116. Oxford University Press, Oxford.
- Pierce, N.E. & Eastal, S. (1986) The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glauropsyche lydamus*. *Journal of Animal Ecology*, **55**, 451–462.
- Pierce, N.E. & Elgar, M.A. (1985) The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behavioral Ecology and Sociobiology*, **16**, 209–222.
- Pierce, N.E., Kitching, R.L., Buckley, R.C., Taylor, M.F.J. & Benbow, K.F. (1987) The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behavioral Ecology and Sociobiology*, **21**, 237–248.
- Pierce, N.E. & Mead, P.S. (1981) Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science*, **211**, 1185–1187.
- Pierce, N.E. & Nash, D.R. (1999) The imperial blue *Jalmenus evagoras* (Lycaenidae). *Biology of Australian Butterflies – Monographs on Australian Lepidoptera* (ed. by R. L. Kitching, E. Scheermeyer, R. E. Jones and N. E. Pierce), CSIRO Publishing, Canberra.
- SAS Institute Inc. (1995) *JMP® User's Guide, Version 3.1*. SAS Institute Inc., Cary, North Carolina.
- Schönrogge, K., Wardlaw, J.C., Thomas, J.A. & Elmes, G.W. (2000) Polymorphic growth rates in myrmecophilous insects. *Proceedings of the Royal Society of London, Series B*, **267**, 771–777.
- Seifert, B. (1996) *Ameisen: Beobachten, Bestimmen*. Naturbuch-Verlag, Augsburg, Germany.
- Seufert, P. & Fiedler, K. (1996) The influence of ants on patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. *Oecologia*, **106**, 127–136.
- Sibatani, A., Saigusa, T. & Hirowatari, T. (1994) The genus *Maculinea* van Eecke, 1915 (Lepidoptera: Lycaenidae) from the East Palaearctic Region. *Tyô to Ga*, **44**, 157–220.
- Smiley, J.T., Atsatt, P.R. & Pierce, N.E. (1988) Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. *Oecologia*, **76**, 416–422.
- Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*, 3rd edn. W. H. Freeman, New York.
- Stoltze, M. (1996) *Danske Dagsommerfugle*. Gyldendal, Copenhagen.

- Thomas, J.A. (1984) The behaviour and habitat requirements of *Maculinea nausithous* (the dusky large blue butterfly) and *M. teleius* (the scarce large blue) in France. *Biological Conservation*, **28**, 325–347.
- Thomas, J.A. (1995) The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. *Ecology and Conservation of Butterflies* (ed. by A. S. Pullin), pp. 180–197. Chapman & Hall, London.
- Thomas, J.A., Clarke, R.T., Elmes, G.W. & Hochberg, M.E. (1998a) Population dynamics in the genus *Maculinea* (Lepidoptera: Lycaenidae). *Insect Populations* (ed. by J. P. Dempster and I. F. G. MacLean), pp. 261–290. Chapman & Hall, London.
- Thomas, J.A. & Elmes, G.W. (1998) Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecological Entomology*, **23**, 457–464.
- Thomas, J.A. & Elmes, G.W. (2001) Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proceedings of the Royal Society of London, Series B*, **268**, 471–477.
- Thomas, J.A., Elmes, G.W. & Wardlaw, J.C. (1998b) Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proceedings of the Royal Society of London, Series B*, **265**, 1895–1903.
- Thomas, J.A., Elmes, G.W., Wardlaw, J.C. & Woyciechowski, M. (1989) Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia*, **79**, 452–457.
- Thomas, J.A. & Wardlaw, J.C. (1990) The effect of queen ants on the survival of *Maculinea arion* larvae in *Myrmica* ant nests. *Oecologia*, **85**, 87–91.
- Thomas, J.A. & Wardlaw, J.C. (1992) The capacity of a *Myrmica* ant nest to support a predacious species of *Maculinea* butterfly. *Oecologia*, **91**, 101–109.
- Thompson, J.N. (1999) Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, **153**, S1–S14.
- Tolman, T. & Lewington, R. (1997) *Butterflies of Britain and Europe*. Harper Collins, London.
- Wahlund, S. (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas*, **11**, 65–106.
- Walén, L., Seppä, P. & Sundström, L. (2001) Reproductive allocation within a polygynous, polydomous colony of the ant *Myrmica rubra*. *Ecological Entomology*, **26**, 537–546.
- Wardlaw, J.C. & Elmes, G.W. (1996) Exceptional colony size in *Myrmica* species (Hymenoptera: Formicidae). *Entomologist*, **115**, 191–196.
- Wardlaw, J.C., Elmes, G.W. & Thomas, J.A. (1998) Techniques for studying *Maculinea* butterflies. II. Identification guide to *Myrmica* ants found on *Maculinea* sites in Europe. *Journal of Insect Conservation*, **2**, 119–127.
- Wynhoff, I. (1998) The recent distribution of the European *Maculinea* species. *Journal of Insect Conservation*, **2**, 15–27.
- Yamaguchi, S. (1988) *The Life Histories of Five Myrmecophilous Lycaenid Butterflies of Japan*. Kodansha, Japan.
- Zar, J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey.

Accepted 2 December 2001