

Increased genetic diversity as a defence against parasites is undermined by social parasites: *Microdon mutabilis* hoverflies infesting *Formica lemani* ant colonies

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Genetic diversity can benefit social insects by providing variability in immune defences against parasites and pathogens. However, social parasites of ants infest colonies and not individuals, and for them a different relationship between genetic diversity and resistance may exist. Here, we investigate the genetic variation, assessed using up to 12 microsatellite loci, of workers in 91 *Formica lemani* colonies in relation to their infestation by the specialist social parasite *Microdon mutabilis*. At the main study site, workers in infested colonies exhibited lower relatedness and higher estimated queen numbers, on average, than uninfested ones. Additionally, estimated queen numbers were negatively correlated with estimated average numbers of mates per queen within infested colonies. At another site, infested colonies also exhibited significantly lower worker relatedness, and estimated queen numbers were comparable in trend. In contrast, in two populations of *F. lemani* where *M. mutabilis* was absent, relatedness within colonies was high (40 and 90% with $R > 0.6$). While high genetic variation can benefit social insects by increasing their resistance to pathogens, there may be a cost in the increased likelihood of infiltration by social parasites owing to greater variation in nestmate recognition cues. This study provides the first empirical test of this hypothesis.

Keywords: myrmecophily; microsatellites; polygyny; monogyny; social parasites; colony gestalt

1. INTRODUCTION

It is widely accepted that high levels of genetic variation maintain flexibility in a species' response to a changing environment, including disease, parasite and predator pressures. It is also recognized that animals living at high densities and in social groups are especially vulnerable to parasites and disease (Alexander 1974), as the aggregation of highly related individuals increases the chance of transmission and infection (e.g. Brown & Brown 1986). In contrast to this, social animals achieve advantages over non-social species through the co-operation of closely related individuals within family groups, leading to a potential conflict between the benefits of social living and vulnerability to disease.

Mechanisms that increase genetic variation within the colonies of most ants (and other social hymenoptera) include the occurrence of multiple functional queens (polygyny) and the multiple mating of queens (polyandry). Both can lead to another set of within-colony conflicts over resource allocation or reproduction, with a trade-off between producing more sexual siblings and lower relatedness between these siblings. In some species, polyandry and polygyny may be negatively correlated, suggesting that an optimum level of genetic diversity may

be selected for (Keller & Reeve 1994). However, Schmid-Hempel & Crozier (1999) found no overall relationship between the number of queens and mates in ants once phylogeny was accounted for, and the pattern may vary within species and between populations (Keller & Reeve 1994).

Thus, individuals within colonies of social insects are often less related than was once predicted. Moreover, several studies found that colonies containing close relatives are less fit and less productive than those that contain distantly related individuals (for ants: Sundström 1995; Cole & Wiernasz 1999; Hughes & Boomsma 2004; but see Fjerdingstad *et al.* 2003). Hypotheses to explain why hymenoptera are polygynous or polyandrous (Page 1986; Sherman *et al.* 1988; Boomsma & Ratnieks 1996) suggest that genetic diversity allows the expression of a more complete genetically based caste system, as well as expanding the environmental tolerances of a species, reducing the proportion of diploid males and allowing the enhancement of queens' inclusive fitness to induce workers to produce males. In addition, increased genetic diversity is thought to be adaptive (Page 1986; Crozier & Fjerdingstad 2001), where productivity is reduced due to susceptibility to parasites (Hamilton 1987; Sherman *et al.* 1988; Schmid-Hempel 1998). Evidence supporting this idea comes from bumble- and honeybees (Liersch & Schmid-Hempel 1998; Baer & Schmid-Hempel 1999; Tarpay 2003; Tarpay & Seeley 2006), from comparative studies involving large numbers of social insect host species and their associated parasite species (Schmid-Hempel & Crozier 1999), and from variation in colonies of the ant *Acromyrmex*

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octospinosus, both to resist a virulent fungal parasite (Hughes & Boomsma 2004) and to hinder the ability of the parasites to adapt while cycling within genetically diverse colonies (Hughes & Boomsma 2006).

Most evidence to date for the genetic variation versus parasitism hypothesis comes from studies of parasites and pathogens that attack individuals within colonies. However, generalizations have also been made for parasites that possess very different life histories. For example, the viruses, bacteria, microsporidia, macro-parasites, parasitoids and social parasites assayed by Schmid-Hempel (1994) employ a diversity of mechanisms for infesting insect societies—ranging from the antigenic surface protein recognition modes of pathogens to the chemical mimicry used by some myrmecophilous arthropods (Akino *et al.* 1999; Lenoir *et al.* 2001)—and it is not self-evident that a simple relationship between genetic variability and resistance applies across all types of interactions (Akino *et al.* 1999; Lenoir *et al.* 2001; van Baalen & Beekman 2006). Interactions between social parasites and their hosts differ fundamentally from those of pathogens, since the former infiltrate insect societies to exploit the resources of the colony as a whole, whereas the latter parasitize individual ants (Thomas *et al.* 2005). In the few social parasites studied, successful infiltration of the host society involves chemical camouflage or mimicry of host-recognition signals (Hölldobler & Wilson 1990; Akino *et al.* 1999; Lenoir *et al.* 2001; Schönrogge *et al.* 2004). The increase of genetic diversity within highly polygynous–polydomous social insect species may increase resistance to colony pathogens, but it might simultaneously make these societies penetrable by social parasites. Here, we test this latter hypothesis through studies of the ant *Formica lemani* and its syrphid social parasite, *Microdon mutabilis*. We compare the genetic variation between infested and uninfested colonies in two *F. lemani* populations where *M. mutabilis* occurs, and contrast these with two other *F. lemani* populations with no record of *M. mutabilis* parasitization.

2. MATERIAL AND METHODS

(a) Study system

Little is known of the genetics or demography of *F. lemani* except that it is at least facultatively polygynous and polydomous, and is more apt to form colonies by budding than the closely related *Formica fusca* (Collingwood 1979). *Microdon mutabilis* lives almost its entire life cycle within *F. lemani* nests, where the larvae are predators of ant brood (Elmes *et al.* 1999; Schönrogge *et al.* 2002). Like many social parasite species, *M. mutabilis* is rare in comparison with its host (Schönrogge *et al.* 2002), and shows extreme host specificity not simply to one species of ant but to individual host populations and possibly to particular family groups within a *F. lemani* population (Elmes *et al.* 1999). In this sense, the *F. lemani*–*M. mutabilis* host–parasite interaction is analogous to some highly host-adapted systems of true parasites (e.g. Dybdahl & Lively 1996).

(b) Field collections

Our main infested study site was on the Isle of Mull in Scotland (NM 518 463 UK grid), where, in May 2001, 267 *F. lemani* colonies were identified under stones on either side of a 4 km track within a forest. Ant colonies were found

continuously in the cleared area about 2 m on either side of the road but not within the forest. From 41 of these colonies (ranging from 1.8 to 3982 m apart), we collected 1952 workers (mean 47.6 workers per colony $\pm$ s.e. 2.6) and 26 queens (0–8 per colony). A second infested population was studied on the Burren, an area of limestone pavement in Ireland (M2708 Irish grid). Here, we collected 1394 workers (45.07 ± 3.89) and 32 queens (0–8 per colony) from 30 colonies (2–517 m apart) on a 500 m $\times$ 500 m grid in 2002, representing all the colonies within the grid. In 2002, we collected *F. lemani* from two well-known sites where *M. mutabilis* has never been recorded: 377 workers (37.7 ± 2.07) were taken from 10 colonies (4.0–188.7 m apart) from coastal heath in St. Agnes (StAg), Cornwall (SW 699 502 UK grid); and 358 workers (35.8 ± 2.16) from 10 colonies (3.1–74.8 m apart) in a field with limestone outcrops near Limerick (Lim), Ireland (R2951 Irish grid). Nests located under stones within 1 m of each other, and with inter-connecting tunnels, were considered to be from the same colony. Ants were stored in 96% ethanol until genetic analysis.

(c) Evidence for parasitism

Nests were examined in May when larvae of *M. mutabilis* move to the top of the nests to pupate. About 60% of nests were excavated to detect immature larvae.

(d) Microsatellite analyses

DNA was extracted from whole individual workers using a modified Chelex 100 method (Walsh *et al.* 1991). Individual ants were screened for variation at 12 microsatellite loci (FL12 from Chapuisat (1996); 11 FE loci from Gyllenstrand *et al.* (2002), see Table 1 in the electronic supplementary material). Seven of these loci were separately amplified (see Table 1 in electronic supplementary material) using standard Bioline Taq polymerase, while the remaining five co-amplified using the Qiagen Multiplex kit (Qiagen). Samples from the Burren, StAg and Lim were amplified with a subset of the most variable nine loci (see Table 1 in the electronic supplementary material). The 5' end of each forward primer was fluorescently labelled with 6-FAM, HEX or TET (see Table 1 in the electronic supplementary material) to enable detection by a MJ Research Basestation automatic genotyping machine. Amplification conditions (see Table 1 in the electronic supplementary material) for the singly amplified loci were: initial denaturation at 95°C for 5 min, then cycles of denaturation at 94°C for 45 s, annealing for 45 s, extension for 1 min at 72°C followed by a final extension for 10 min at 72°C. Conditions for the multiplex amplifications were: initial denaturation for 15 min at 95°C followed by 32 cycles of 94°C for 30 s, 57°C for 90 s, 72°C for 1 min with a final extension of 60°C for 30 min. Products from all singularly amplified reactions were mixed equally (2.5 μ l each) and then diluted 1/3 with sigma water. One microlitre of each dilution was then mixed with 4 μ l formamide and 0.17 μ l ROX 400HD (Applied Biosystems) size standard per well for electrophoresis. Multiplex products were treated similarly, except that they were diluted 1/30. A sample of 1.5 μ l from each mixed loci sets was electrophoresed for 90 min at 40°C using the supplied KBB buffer system (MJ Research). The gel results were analysed with CARTOGRAPHER software (MJ Research).

(e) Marker variation

The Mull population was tested for deviations from Hardy–Weinberg proportions, using the program GENEPOP v. 3.1 (Raymond & Rousset 1995), by examining the genotype frequency distributions and using one individual from each colony. Exact p -values were estimated by the Markov chain method implementing 1000 dememorization steps, 1000 batches and 1000 iterations per batch for all tests. Global tests across loci were constructed using Fisher's combined probability method as implemented in GENEPOP. Genotypic linkage disequilibrium among alleles was also tested using GENEPOP and significance levels adjusted using sequential Bonferroni corrections (Hochberg 1988). Null allele frequencies were estimated using MICRO-CHECKER (Van Oosterhout et al. 2004) on a subset of workers that amplified for all loci in order to disregard possible errors due to degraded DNA. Observed heterozygosity per locus was determined using the MICROSATELLITE TOOLKIT (Park 2001).

(f) Population substructuring

The F statistic for the effect of subpopulations compared to the total population (F_{st}) was calculated, and its significance tested, to assist in the evaluation of loci by testing the hypothesis that there was population substructuring on Mull. These data were arranged into individuals within colonies within the total population for the Mull site only and the tests were implemented in FSTAT v.2.9.3.2 (Goudet 2001).

(g) Relatedness estimations

Relatedness estimates (R) were calculated from worker and queen genotypes using the program RELATEDNESS v. 5.08 (Goodnight & Queller 1998), which implements the methods described by Queller & Goodnight (1989). Colonies were weighted equally and standard errors were estimated by jackknifing over loci. Background allele frequencies for the relatedness estimates for each site were calculated separately using data from all genotyped workers at each site. Relatedness values between infested and uninfested colonies were compared using $R-R'$ unpaired difference, with p -values estimated from jackknifing over loci, in RELATEDNESS.

(h) Estimates of queen and mate numbers

Individual worker genotypes were used as input for the maximum likelihood program COLONY (Wang 2004) to estimate the number of queens and their mates contributing to the workers, per colony, at each site. Allelic dropout and other errors were assumed to be 5%. As only one sex is able to be designated as multiply mating in the program, we expected queens to be the polygamous sex with males expected to be monogamous. We compared the number of queens, estimated for uninfested and infested colonies, using a generalized linear model with Poisson errors and a logit link implemented in SPLUS v. 7.0. Since the variance is over-dispersed, significance was assessed on the deletion of terms using an F -test. Similarly, we compared the average number of mates per queen per colony (see §3). For simplicity, standard errors in text and error bars in the figure are calculated on untransformed data, which makes little practical difference. We did not estimate the genetically effective queen number (Pedersen & Boomsma 1999) as the number of mates per queen was estimated to be high (see §3) and thus the assumption of singly mated queens was invalidated. In any case, comparisons of the R estimates



Figure 1. Average relatedness (R) of *Formica lemani* ants on Mull (with standard errors) determined using 12 micro-satellite loci. Comparisons are within or between workers and queens within *Microdon mutabilis* infested (grey) and uninfested (black) colonies. All differences were highly significant (see text).

above give a comparable test of genetic diversity. A bivariate Pearson's correlation was used to test whether a relationship existed between the estimates of queen numbers and the average number of mates per queen at each site. Additionally, infestation levels of infested colonies only were compared to R estimates using a bivariate Pearson's correlation in SPSS.

3. RESULTS**(a) Infestation**

Of the 267 *F. lemani* colonies on Mull, 42 (18.7%) were parasitized by *M. mutabilis*. Of the 41 colonies sampled for genetic analysis, 22 (54%) were infested (mean number $\pm$ s.e.: larvae 0.40 ± 0.22 , fresh pupae 3.70 ± 1.22 , old pupal cases 5.01 ± 2.80) and 19 (46%) were uninfested. The Burren site was significantly (Fisher's exact two-tailed $p < 0.0001$) more heavily infested as compared with the surveyed Mull colonies, with 20 (66%) colonies containing *M. mutabilis* (mean number $\pm$ s.e.: larvae 1.70 ± 0.57 , fresh pupae 2.05 ± 0.76 , old pupal cases 2.85 ± 0.66) and 10 (33%) not. No colony from StAg or Lim was infested.

(b) Relatedness

Information on variation of the loci used can be found in the electronic supplementary material to this paper. For all Mull colonies, the average value of within-colony relatedness, R , between workers was 0.244 ± 0.015 s.e. The average relatedness of workers within infested colonies on Mull was significantly lower than in uninfested colonies (jackknife over 12 loci: $R-R'$ difference = -0.163 ; s.e. = 0.021 ; $p < 0.0001$; figure 1). Similarly, the average R value of queens to workers was significantly lower (jackknife over 12 loci: $R-R'$ difference = -0.128 ; s.e. = 0.046 ; $p < 0.0001$) within infested Mull colonies than within the uninfested ones (figure 1). Finally, the average queen to queen R value on Mull was also significantly lower (jackknife over 12 loci: $R-R'$ difference = -0.175 ; s.e. = 0.098 ; $p < 0.0001$) in infested colonies than in uninfested ones (figure 1), although this comparison is based on a very small sample (multiple queens were collected from two uninfested colonies, containing three or eight queens, and two infested colonies each containing three queens).

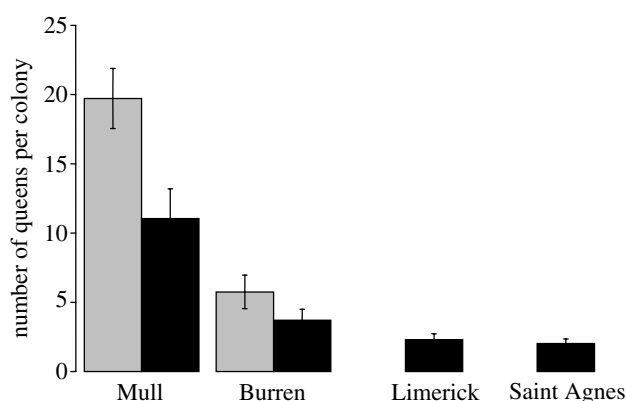


Figure 2. Estimated queen numbers of *Formica lemani* (with standard errors) from each site within *Microdon mutabilis* infested (grey) and uninfested (black) colonies. The comparison between Mull infested and uninfested colonies was highly significant; the uninfested populations at St Agnes and Limerick did not differ significantly, and both contained significantly fewer estimated queens per colony than the infested populations (Mull and Burren) (see text).

The same strong pattern was found at our second infested site, the Burren. The average R value among workers within all colonies was 0.492 ± 0.017 s.e. Significant differences existed between the relatedness of workers within infested (mean $R = 0.458 \pm 0.019$ s.e.) and uninfested (0.565 ± 0.024) Burren colonies (jackknife over nine loci: $R - R'$ difference = -0.108 ; s.e. = 0.026 ; $p < 0.0001$). Similarly, the average R value of queens to workers was significantly lower (jackknife over nine loci: $R - R'$ difference = -0.367 ; s.e. = 0.124 ; $p < 0.0001$) within infested Burren colonies than within uninfested ones. However, this comparison is based on a small sample from uninfested colonies (two colonies with one sampled queen each), and no queen to queen comparison could therefore be made. The average within-colony worker relatedness in the *M. mutabilis* uninfested populations was 0.49 ± 0.029 and 0.77 ± 0.034 for Lim and StAg, respectively.

(c) Queen and mate numbers

On Mull, the estimated average number of queens per infested colony was significantly greater ($F_{1,40} = 7.73$, $p = 0.008$) than that of uninfested colonies (figure 2). There was no difference ($F_{1,40} = 0.057$, $p = 0.81$) in the average number of mates per queen (infested, 1.69 ± 0.14 s.e.; uninfested, 1.76 ± 0.23). Interestingly, there was a significantly negative correlation (Pearson correlation, $r_{22} = -0.556$, two-tailed $p = 0.007$) between the estimated queen number and the average number of mates per queen in infested colonies. The same comparison among uninfested colonies from Mull was not significant ($r_{19} = -0.207$, $p = 0.396$). The linear regression with log-transformed estimates for queens and mates clearly demonstrates this difference (figure 3).

Infested colonies on the Burren also contained a greater estimated average number of queens than uninfested colonies (figure 2), although the difference is not significant ($F_{1,29} = 1.45$, $p = 0.24$), perhaps due to the small number of uninfested colonies located there. The estimated average number of mates per queen per colony did not differ significantly ($F_{1,29} = 0.74$, $p = 0.4$) between the infested (1.96 ± 0.227) and uninfested (1.68 ± 0.138)

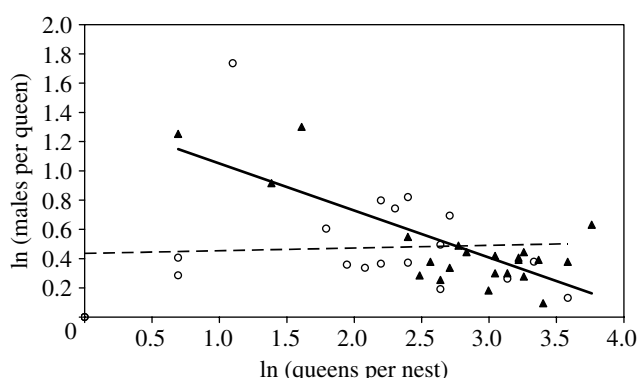


Figure 3. A linear regression of log-transformed $[\ln(x)]$ estimated number of queens (polygyny) and estimated number of mates (polyandry) in *F. lemani* Mull colonies infested by *M. mutabilis* (solid triangles, solid line) and without *M. mutabilis* (open circles, dashed line). For infested nests the correlation is highly significant ($F_{1,20} = 29.6$, $p < 0.001$), while in non-infested nests it is not ($F_{1,17} = 0.04$, $p > 0.8$).

Burren colonies. No significant correlations between queen and mate estimates were found for either infested ($r_{20} = -0.073$, $p = 0.761$) or uninfested ($r_{10} = 0.088$, $p = 0.809$) Burren colonies. Correlations between queen and mate numbers at Lim ($r_{10} = 0.189$, $p = 0.601$) and StAg ($r_{10} = -0.341$, $p = 0.335$) were also not significant.

There was no significant difference between Lim and StAg for either estimated queen numbers (figure 2, $F_{1,19} = 0.38$, $p > 0.05$) or average estimated mate numbers ($F_{1,19} = 1.39$, $p > 0.05$; Lim mean = 2.03 ± 0.321 ; StAg mean = 1.52 ± 0.294) per colony. When the Lim and StAg data were combined, all queen number comparisons with either Mull infested ($F_{1,41} = 111.02$, $p < 0.0001$), Mull uninfested ($F_{1,38} = 31.02$, $p < 0.0001$), Burren infested ($F_{1,39} = 12.0$, $p < 0.01$) or Burren uninfested ($F_{1,29} = 6.11$, $p < 0.05$) colonies were significantly lower. Similar comparisons with average mate number per queen per colony were not significant (all $p > 0.55$: Lim/StAg versus Mull infested, $F_{1,41} = 0.094$; Mull uninfested, $F_{1,38} = 0.002$; Burren infested, $F_{1,39} = 0.357$; Burren uninfested, $F_{1,29} = 0.084$).

(d) Correlations with infestation levels

No significant correlations were found between R and the number of old pupae, current pupae or combined infestation levels (Pearson's correlation $r_{22} = 1.09$, $p = 0.315$; $r_{22} = -0.08$, $p = 0.362$; $r_{22} = 0.06$, $p = 0.396$, respectively) in Mull colonies. Similarly, for the Burren samples, we found no significant correlations of R with old pupae ($r_{20} = 0.111$, $p = 0.64$), present pupae ($r_{20} = -0.172$, $p = 0.467$) or combined pupae count ($r_{20} = -0.071$, $p = 0.765$). As we did not estimate the size of colonies, we were unable to factor this into our comparisons of R and infestation levels, limiting our ability to detect existing relationships.

4. DISCUSSION

Our results clearly indicate that populations and individual colonies of *F. lemani* that are genetically diverse are more likely to be infested with *M. mutabilis* than the homogeneous ones. Although our findings are correlational, they are in stark contrast to the negative relationship between relatedness and susceptibility

reported for somatic parasites and pathogens (e.g. Baer & Schmid-Hempel 1999; Tarpay 2003; Hughes & Boomsma 2004; Tarpay & Seeley 2006). In the case of pathogens, resistance can be chemical or involve behaviour that suppresses transmission, such as allogrooming, 'dead worker removal', and general nest hygiene; and it is increasingly evident that a lack of genetic diversity in the innate immune response promotes pathogen/parasite success in highly related ant colonies (Rolf & Siva-Jothy 2003). In contrast, most known examples of *ca* 10 000 species of ant social parasite employ subterfuge (mimicry, camouflage and stealth) to penetrate host societies (Hölldobler & Wilson 1990; Thomas *et al.* 2005), and while resistance via the immune response against pathogens relies on a lock-and-key recognition, resistance to myrmecophile attack depends on the ability of worker ants to detect differences between an intruder's chemical signature and the mean chemical template of the colony to which the workers belong (Vander Meer & Morel 1998).

In most insect societies, species-, nestmate- and in some cases kin-recognition are primarily based on their members' ability to detect characteristic cocktails of surface lipids, often referred to as cuticular hydrocarbons (CHCs), on fellow members (Crozier 1987; Hölldobler & Wilson 1990; Hannonen & Sundström 2003). The origin of CHCs is still under debate but has been demonstrated to have a genetic component in some insect species (*Drosophila melanogaster*: Coyne *et al.* 1999; Takahashi *et al.* 2001). In ants, CHCs have been shown to be largely inherited, for example, in *Formica* (Beye *et al.* 1998), and to be under selection, as shown by certain invasive species (Nowbahari *et al.* 1990; Tsutsui *et al.* 2000; Boomsma *et al.* 2003). At the same time, environmental factors such as diet may modify CHC signatures (e.g. Provost 1989; Elmes *et al.* 2004). The cue-template model (Vander Meer & Morel 1998) hypothesises that each worker in a colony produces a distinctive chemical signature, providing a baseline against which the odours of other individuals are assessed. In polygynous ants (like *F. lemani*), the less closely related colony members exchange CHCs by allogrooming, and the template that workers accept is a mean signature for the whole nest, known as the colony gestalt odour (Crozier & Dix 1979; Crozier 1987; Vander Meer 1998). As a result, the colony gestalt odour in monogynous species or colonies tends to be tighter and better defined than that in polygynous species or colonies.

The more diffuse the colony gestalt odour (owing to genetically diverse workers), the greater the variance around the mean template that workers have to tolerate if they are not to exclude nest mates (Starks *et al.* 1998). In theory, this makes a cosmopolitan ant society more susceptible to intruders, even if they are imperfect mimics of the colony's broad gestalt odour. Although this hypothesis is only intended for *F. lemani*, it may also apply to other *Serviformica* species and indeed, social insects in general.

In practice, it has long been recognized that social parasite species are usually associated with polygynous and polydomous ant species (Buschinger 1970; Alloway *et al.* 1982; Thomas *et al.* 2005). Among the few social parasites whose secretions have been analysed, *Maculinea* butterflies do indeed synthesize CHCs that mimic those of their host's societies so closely that the worker ants rescue

and feed the parasitic caterpillars in preference to their own larvae (Akino *et al.* 1999; Nash *et al.* 2002; Schönrögge *et al.* 2004; Thomas *et al.* 2005).

The exact mechanism used by *M. mutabilis* to infiltrate *F. lemani* colonies is unknown, but it undoubtedly involves camouflage and probably mimicry (Elmes *et al.* 1999; Napper 2004). The four populations we studied represent a range of biotopes: coastal heath in StAg; a grassy roadside on Mull; limestone outcrops in Lim; and the largest limestone pavement in Europe (Burren). Using the populations where *M. mutabilis* is absent (StAg and Lim) as a baseline, we suggest that *F. lemani* populations generally consist of colonies that have low levels of polygyny, with a substantial proportion being monogynous. Thus, not only do the nests on the Burren and Mull contain significantly higher levels of polygyny than at Lim and StAg, but within the Mull and Burren populations, colonies attacked by *M. mutabilis* were more polygynous than unparasitized ones. Although other explanations may explain the lower genetic diversity and lack of parasites at the two uninfested sites, our intrapopulation comparison on both Mull and the Burren provides compelling evidence for a relationship between *M. mutabilis* infestation and genetic diversity.

Slave-making ants provide the only comparable well-studied system in which the degree and distribution of polygyny in a host ant may be affected at the population level by an intruder. However, this involves a different interaction in which slave-making workers release powerful alarm pheromones during raids that cause the host ants to attack each other. This diminishes the latter's ability to protect their brood, which is captured and reared as slaves in the slave-makers' colonies. In at least one system (raider, *Harpagoxenus sublaevis*; host, *Leptothorax acervorum*), monogynous host colonies are better able to resist slave-making raids (Foitzik *et al.* 2003). Furthermore, *Leptothorax longispinosus* (host) colonies in the vicinity of *Protomognathus americanus* (slave-maker) colonies are less polygynous, have higher relatedness and also fewer workers compared with host colonies away from slave-makers. The fact that slave colonies contain host workers which are genetically more diverse than workers in the neighbouring colonies may simply result from raids on multiple-host colonies.

The similarities between these studies and ours are intriguing, although in both systems the cause and the effect are unclear. Slave-makers might raid genetically diverse colonies more easily, leaving monogynous or weakly polygynous colonies as survivors in areas where the slave-makers occur. Alternatively, the slave-makers may preferentially settle in areas that have small monogynous colonies of their hosts (Foitzik *et al.* 2003).

The hypothesis that only genetically diverse colonies and populations of *F. lemani* can be infested by *M. mutabilis*—because it is easier for the parasite to enter colonies whose odour diversity is great—is not the only explanation of our results. Another non-exclusive possibility is that the lower relatedness of infested nests is a consequence of being parasitized. In this scenario, colonies become infested with *M. mutabilis* and subsequently acquire additional queens and/or recruit workers from neighbouring nests to minimize the impact of the parasite. We have often found that the same host nest is attacked in three or more consecutive years, as

indicated by the simultaneous presence of old pupal cases, fresh pupae and young biennial larvae of *M. mutabilis* in the same nest (Schönrogge *et al.* 2006). In these nests, worker numbers must inevitably decline or be compensated, owing to the large amounts of ant brood eaten by *M. mutabilis* (Schönrogge *et al.* 2000, 2006). Extra-nest queens can be adopted to increase a colony's ergonomic efficiency (Oster & Wilson 1978) by either increasing its work-force size or increasing the genetic diversity of the colony as a defence against strong parasitism (Rosengren *et al.* 1993).

Our finding of a negative correlation between estimated queen numbers and average numbers of mates per queen suggests that, at least on Mull, these two pathways to increased genetic diversity, polygyny and polyandry do not operate independently, suggesting that there may some other benefit to polyandry/polygyny distinct from its implications for infection by *Microdon*. There would be no relationship between polygyny and polyandry if colonies adopt queens in order to combat the presence of the parasite, as colonies would not have the ability to increase the numbers of mates of their queen(s). Thus, the association between genetic diversity and *Microdon* infestation is more likely to be due to genetically diverse nests being more susceptible to invasion rather than to infested colonies adopting additional queens.

We suggest that a negative correlation between estimated queen numbers and average numbers of mates per queen could arise in two ways: (i) for the Mull population at least, there may a trade-off between susceptibility to *Microdon* and resistance to pathogens. To date there is no information about pathogen pressures on *F. lemani* colonies, but if colonies with low queen numbers tend to be more polyandrous, and selection is strong throughout the population, pathogen exposure could determine which *F. lemani* populations are susceptible to *Microdon* attacks; (ii) we recently found that *F. lemani* colonies infested by *Microdon* produce about twice as many queens per worker compared with uninfested ones (Schönrogge *et al.* 2006). The fate of new queens produced in infested nests is currently unknown. However, the decrease in mate numbers with increasing numbers of queens in infested colonies could arise through a higher inclination to swarm by these new queens, leading to multiple mating and subsequent colony founding, either independently or in small groups. This would also be consistent with the lack of such a correlation among non-infested colonies. Clearly, the population level impact by *M. mutabilis* on its host *F. lemani* warrants further investigation.

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