

Use of population genetic data to infer oviposition behaviour: species-specific patterns in four oak gallwasps (Hymenoptera: Cynipidae)

Rachel J. Atkinson^{1*}, Gilean A. T. McVean² and Graham N. Stone³

¹Department of Biology, University of Stirling, Stirling FK9 4LA, UK

²Department of Statistics, University of Oxford, 1 South Parks Road, Oxford OX1 3TG, UK

³ICAPB, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK

Many species of oak gallwasp (Hymenoptera: Cynipidae: Cynipini) induce galls containing more than one larva (multilocular galls) on their host plant. To date, it has remained unclear whether multilocular galls result solely from clustered oviposition by a single female, or include the aggregated offspring of several females (multiple founding). We have developed a novel maximum-likelihood approach for use with population genetic data that estimates the number and genotypes of parents contributing to offspring from each gall. We apply this method to allozyme data from multiple populations of four oak gallwasps whose asexual generations develop in multilocular galls (*Andricus coriarius*, *A. lucidus*, *A. panteli* and *A. seckendorffi*). We find strong evidence for multiple founding in all four species, and show the data to be compatible with multiple founding rather than founding by a single foundress mated with multiple males. The extent of multiple founding differs among species: in *A. lucidus* and *A. seckendorffi* most galls are induced by a single female, whereas in *A. coriarius* and *A. panteli* over half of the galls sampled were multiple founded. We suggest that variation in levels of multiple founding may be due to consistent ecological differences between the four species.

Keywords: superparasitism; gallwasp; multilocularity; multiple founding; Cynipidae; oviposition

1. INTRODUCTION

One of the most distinctive features of oak gallwasps (Hymenoptera: Cynipidae: Cynipini) is the ability to induce galls on their host (Askew 1984; Stone *et al.* 2002). The gall provides food and protection for the developing gallwasp larva and is initiated following oviposition by a female into a specific tissue type on the host tree (usually an oak within the subgenus *Quercus*). The phenotype of the gall is species and generation specific and results from two levels of interaction between the gallwasp and the oak (Stone & Cook 1998). First, ovipositional decisions made by the female determine the host plant, the organ on which the gall develops (leaf, bud, root etc.), and whether the gall contains a single larva (unilocular galls) or many larvae (multilocular galls). Second, once the eggs hatch, the gallwasp larvae secrete as yet uncharacterized morphogens that induce gall formation by the oak tissues (Schönrogge *et al.* 2000).

Multilocular galls are widespread in gall-inducing insects, including all five tribes of gall-inducing cynipids (Folliot 1964; Askew 1984; Stille 1985), tephritid gall flies (Eber & Brandl 1994; Abrahamson & Weis 1997) and cecidomyiid gall midges (Weis *et al.* 1988). Detailed studies of these taxa suggest that the principle selective pressure determining the gall chamber number is the high mortality rate inflicted by natural enemies, in particular chalcid parasitoids (Weis *et al.* 1983; Jones 1983; Kato & Hijii 1993; Zwolfer & Arnold-Rinehart 1994; Stone *et al.* 2002). Chalcid parasitoids oviposit into or onto their hosts

by penetrating gall tissues with a drilling ovipositor. The more chambers there are in a multilocular gall, the larger the gall and the greater the proportion of larvae that are beyond the reach of parasitoid attack (Jones 1983; Stille 1984; Price & Clancy 1986; Latto & Briggs 1995; Freese & Zwolfer 1996). Assuming a monotonic relationship between gall size and protection against parasitism, in the absence of any other factors a female could maximize her offspring's survival by laying all her eggs in one location, as observed in the rose gallwasp *Diplolepis rosae* (Bronner 1985).

Although this strategy may reduce the rate of parasitoid attack per larva, the concentration of a female's entire reproductive success in a single location might leave her vulnerable to unpredictable events that could destroy the gall (Stille 1984). In addition, a female may risk predation while ovipositing and there is evidence that this risk increases with the time spent at one location (Charnov & Skinner 1984; Rossi *et al.* 1992). There is also a suggestion that offspring emerging from galls containing many chambers suffer a reduced fitness compared with those developing within smaller galls (Weis *et al.* 1983). The exact nature of the trade-off between protection from parasitoid attack, avoidance of stochastic catastrophes and the effect on offspring fitness may determine how a female distributes her eggs across the environment (Freese & Zwolfer 1996; Roitberg *et al.* 1999).

It has usually been assumed that a multilocular gall contains the offspring of one foundress, and that gall size results from the oviposition decisions of a single female. But it could also result from oviposition in the same host organ by two or more conspecific females (multiple founding). The potential advantages of multiple founding

* Author for correspondence (rachel.atkinson@stir.ac.uk).

are twofold: first, allowing a female to secure the benefits of large gall size (lower parasitoid attack rates) without contributing all of the eggs necessary to achieve it, and second, avoiding the costs associated with laying all her eggs in one place (increased risk of predation, potential loss of total reproductive success). Note that it is important to consider whether larvae in a multilocular gall compete for resources and, if so, whether competition among the offspring of two or more foundresses is more severe than among sibs (Craig *et al.* 2000).

Multiple founding could occur through two routes.

- (i) Females could seek out and use sites in which other females have oviposited. Some hymenopteran parasitoids can detect the eggs of conspecifics (Scholz & Poehling 2000), and as cynipids are derived from parasitoid ancestors (Ronquist 1999), it is possible that female gallwasps also have this ability.
- (ii) Females could be forced to lay gregariously due to a limited availability of gall-induction sites. Females of several species of gallwasp are known to have highly specific oviposition patterns (Askew 1961; Ejlsen 1978), and multiple founding could occur without the female being able to detect prior oviposition.

These issues are important for the population biology of all gall inducers that develop in multilocular galls, and for other biological systems with potential for multiple founding. To date, however, very little is known about the existence and almost nothing about the extent of multiple founding in any multilocular insect gall.

Here, we develop a novel maximum-likelihood approach using multilocus genotype (MLG) data from polymorphic allozyme loci to determine the prevalence of multiple founding in populations of four European oak gallwasp species within the genus *Andricus*: *Andricus coriarius* (Hartig 1843), *Andricus lucidus* (Hartig 1843), *Andricus panteli* (Kieffer 1897), and *Andricus seckendorffi* (Wachtl 1879). All four species are cyclically parthenogenetic (Atkinson 2000), with obligate alternation between a sexual generation and an asexual generation. The multilocular gall in each species results from oviposition by mated females of the sexual generation and contains larvae that will develop into asexual females (Folliot 1964; Stone *et al.* 2002). The method calculates the likelihood of observing a given set of genotypes in a single gall for each of a range of founding scenarios by simulating the genotypes of possible foundresses and their mates given population allele frequencies. It assumes that markers are unlinked and that populations are in both Hardy–Weinberg and linkage equilibria, as observed in all cyclically parthenogenetic *Andricus* species studied (Stone & Sunnucks 1993; Atkinson 2000; Stone *et al.* 2001), but is in any case robust to minor deviations from both. By introducing additional parameters to account for species- and population-level heterogeneity in the factors influencing oviposition behaviour, we can both estimate the distribution of foundress number per gall and test hypotheses about the scale of heterogeneity in this distribution. We favour a maximum-likelihood method over the widely used frequentist approach because of the explicit nature of the model and the ability to compare alternative

hypotheses. We also test the extent to which the model provides an adequate description of the data.

2. MATERIAL AND METHODS

(a) *Biological samples*

The multilocular asexual generation galls of four *Andricus* species were sampled from sites across Europe and Turkey in the late summer and autumn of 1998 and 1999, and the adult insects were reared in an outside insectary in Edinburgh. The study sites for each species were as follows.

(i) *Andricus coriarius*

Florence, Italy (43°46' N, 11°15' E: but note that samples were collected within a 100 km radius of the city), 10 galls; Madenli, Turkey (38°08' N, 31°01' E), 11 galls; Gezende, Turkey (36°32' N, 33°09' E), nine galls.

(ii) *Andricus lucidus*

Sopron, Hungary (47°40' N, 16°35' E), 37 galls; Nantes, France (47°14' N, 1°35' W), 28 galls; Florence, Italy (see *A. coriarius*), 31 galls; Beyşehir, Turkey (37°40' N, 31°43' E) 10 galls; London, UK (51°30' N, 0°10' W), 13 galls.

(iii) *Andricus panteli*

Ağlasun, Turkey (37°39' N, 30°32' E), 17 galls; Azrou, Morocco (33°27' N, 5°14' W), 10 galls.

(iv) *Andricus seckendorffi*

Madenli, Turkey (see *A. coriarius*), 21 galls.

(b) *Allozyme electrophoresis*

All the wasps emerging from each gall were screened using cellulose acetate gel electrophoresis at 13 allozyme loci known to be polymorphic in gallwasps, using methods described in Stone & Sunnucks (1993) and Stone *et al.* (2001). Only a subset of the loci were polymorphic in each study species, three for *A. panteli* and *A. coriarius*, and four for *A. seckendorffi* and *A. lucidus*. The loci screened were as follows, with the species in which they were polymorphic in parentheses: HK (EC 2.7.1.1; *A. seckendorffi*, *A. lucidus*), PGM (EC 2.7.5.1; *A. seckendorffi*, *A. panteli*), 6PGD (EC 1.1.1.44; *A. seckendorffi*, *A. lucidus*, *A. coriarius*), GPI (EC 5.3.1.9; *A. seckendorffi*, *A. lucidus*, *A. coriarius*) and GOT (EC 2.6.1.1; *A. coriarius*), PEPb (EC 3.4.11; *A. panteli*), α GPD (EC 1.1.1.8; *A. lucidus*) and AK (EC 2.7.4.3; *A. panteli*). These systems were run on the buffers described in Richardson *et al.* (1986). HK and PGM were run on Tris–glycine, pH 8.6; 6PGD, GPI and GOT were run on Tris–EDTA–maleate–MgCl₂, pH 8.3; and PEPb, α GPD and AK were run on a phosphate buffer, pH 6.1–6.3.

The following analysis uses data combined across loci to give a MLG for each individual.

(c) *Maximum-likelihood analysis of founding patterns*

Consider a gall from which we have sampled n individuals with the set of MLGs X_i , where X_{ij} is the MLG of individual j from gall i . If we define a mating structure G_i as the set of parental genotypes that contribute to a gall (the combination of matings $M \times P$, where M is the set of maternal genotypes and P the set of paternal genotypes to which she has mated), we can define the likelihood of observing the sampled individuals as

$$L\{X_i\} \propto \sum_j \Pr\{G_j|\theta\} \Pr\{X_i|G_j, \theta\}, \quad (2.1)$$

where θ represents the unknown parameters of genotype frequencies, mating preferences and factors relating to the contribution of multiple females in multiple-founded galls. In other words, the likelihood is the sum over mating structures of the probability of such a mating structure occurring, multiplied by the probability of observing the sampled genotypes given the mating structure. In practice, we only compare the mating structures of multiple founders, each mated once, with single founders mated more than once.

The probability of mating structure G_i occurring is a function of allele frequencies, moments of linkage disequilibrium, population structure, mating preferences and oviposition behaviour. These are parameters we do not know and there is little to estimate all parameters simultaneously from the data. However, given that the genotypes were generated by sexual reproduction, we can approximate the probability that the mating structure G_i occurs by assuming random mating (i.e. linkage and Hardy-Weinberg equilibria), random association of females ovipositing at any one site, and that the observed allele frequencies are the true allele frequencies.

The probability of observing the offspring MLGs given G_i also depends on unknown parameters. In particular, whether each foundress or mate has an equal probability of representation among the individuals sampled from each gall. For simplicity, we assume that MLGs are sampled from the multinomial distribution of the genotype frequencies expected under Mendelian segregation and equal representation of foundresses or mates. This assumption could be tested within the likelihood scheme developed, but is left to future research.

Due to the fact that the number of possible mating structures is so large, the simplest way of estimating the likelihood is through Monte Carlo simulation. That is, for each gall we pick mating structures at random given the model parameters and calculate the likelihood of observing the data. Under this scheme the exact likelihood is approximated by the average as follows:

$$L\{X_i\} \approx \frac{1}{N} \sum_j L\{X_i|G_j\}. \quad (2.2)$$

As the probability of observing genotypes from any given gall is low, most of the simulated mating structures could not have generated the observed MLGs. We have therefore used an importance sampling routine to bias simulation towards compatible mating structures, while correcting for the bias. Parental genotypes are selected one locus at a time, and possible offspring MLGs are compared to the data. If compatible, the next locus is simulated. If incompatible, alleles for the locus are sampled again (with the same frequency as before) until a compatible set is found. The likelihood is then corrected by the inverse of the product of the number of attempts required for each locus. This approach means that rare genotypes can be simulated with reasonable efficiency, although even with the importance sampling method, there were two galls for which no compatible parental mating structures could be found. Simulations are continued until the likelihood converges.

In addition, rather than estimate the number of foundresses or mates for each gall, we fit a parametric distribution. Specifically, we assume a modified Poisson distribution such that the probability of k foundresses or mates is

$$\Pr\{k|\lambda\} = \frac{e^{-\lambda}\lambda^k}{k!(1 - e^{-\lambda})}. \quad (2.3)$$

Table 1. Likelihood ratio test of multiple mating versus multiple founding.

species	population	$L_{MF} - L_{MM}^a$
<i>Andricus coriarius</i>	Madenli (Turkey)	31.99
	Gezende (Turkey)	103.66
<i>A. panteli</i>	Florence (Italy)	15.31
	Ağlasun (Turkey)	11.16
<i>A. lucidus</i>	Azrou (Morocco)	26.98
	Sopron (Hungary)	48.53
<i>A. seckendorffi</i>	Nantes (France)	0.78
	Florence (Italy)	15.99
	Beyşehir (Turkey)	-1.91
	London (UK)	23.15
<i>A. seckendorffi</i>	Madenli (Turkey)	12.25

^a $L_{MF} - L_{MM}$ is the difference in maximum-log likelihood for the models with multiple founding (MF) and multiple mating (MM).

For $k > 0$, where $\lambda/(1 - e^{-\lambda})$ is the average number of females or mates per gall ($\lambda = 0$ implies all galls are produced by a single female who mated once). This distribution was chosen because it reflects the null model in which there is no preference for multiple founding, but that suitable oviposition sites are limited. To estimate λ we calculate the likelihood of each gall for a range of foundresses or mates (1-6) and find the value that maximizes the likelihood

$$L\{X_i|\lambda\} \propto \sum_k \Pr\{k|\lambda\} L\{X_i|k\}. \quad (2.4)$$

The total log likelihood is obtained by the sum of the log likelihoods across galls, populations and species. This approach allows us to consider whether there is significant heterogeneity between species and populations in the average number of foundresses or mates per gall. Specifically, we ask whether allowing a different λ for each species or population provides a significant improvement in likelihood as assessed by the approximation that twice the difference in log likelihood, $2\Delta L$, is approximately χ^2 distributed with degrees of freedom equal to the difference in the number of parameters.

Given the maximum-likelihood parameter estimates, it is possible to find the posterior probabilities for characteristics relating to each gall, such as the set of mating structures that gave rise to the sampled offspring and the number of foundresses per gall. These posterior estimates can then be used to assess the accuracy of the fitted model and to improve our understanding of the population processes that have shaped the data.

3. RESULTS

(a) *Single foundress or multiple foundresses?*

We first wish to ascertain whether the data are more likely under a scenario in which multiple, unrelated females, each mated with a single male, can found single galls, or a scenario in which only a single female ever founds a gall, but that she may have mated with multiple, unrelated males. Considering all populations and species together, we find that multiple-founding events are a far more likely explanation for the data than multiple mating ($\Delta L = 298.3$). This pattern is true for all species, and all but one population (for which ΔL is only 1.9 units); see table 1. We therefore conclude that the data strongly

Table 2. Estimated average number of foundresses per gall and proportion of galls founded by a single female.

species	population	n	estimated average number of foundresses (two-unit support interval)		proportion of galls founded by a single female ^a	
			population	species	population	species
<i>Andricus coriarius</i>	Madenli (Turkey)	11	2.94 (2.11–3.07)	2.62	0.09	0.203
	Gezende (Turkey)	9	2.91 (1.09–3.06)	(2.15–3.20)	0.22	
	Florence (Italy)	10	2.02 ^b (1.46–1.90)		0.30	
<i>A. panteli</i>	Ağlasun (Turkey)	17	2.17 ^b (1.70–2.83)	2.05	0.29	0.415
	Azrou (Morocco)	10	1.82 (1.32–2.64)	(1.68–2.54)	0.54	
<i>A. lucidus</i>	Sopron (Hungary)	37	1.36 (1.18–1.63)	1.36	0.76	0.772
	Rennes (France)	28	1.19 (1.03–1.52)	(1.23–1.51)	0.89	
	Florence (Italy)	31	1.22 (1.07–1.48)		0.77	
	Beyşehir (Turkey)	10	1.51 (1.08–2.58)		0.90	
	London (UK)	13	2.00 (1.57–2.88)		0.54	
<i>A. seckendorffi</i>	Madenli (Turkey)	21	1.10 (1.02–1.31)	—	0.90	—

^a Proportion of galls for which the posterior likelihood for the number of foundresses is greatest for one singly mated female.

^b Underestimate due to a sample containing galls for which no compatible mating was found during simulation.

Table 3. Likelihood ratio tests for heterogeneity in parameters of multiple foundressing.

species	species level: $2\Delta L$ ($\Delta\nu$) ^a	population level: $2\Delta L$ ($\Delta\nu$)
<i>Andricus coriarius</i>		2.36* (2)
<i>A. panteli</i>	51.24** (3)	0.51* (1)
<i>A. lucidus</i>		7.42* (4)
<i>A. seckendorffi</i>		n.a.

^a $\Delta\nu$ is the difference in number of parameters between models.
*n.s., $p > 0.05$. ** $p < 0.001$.

support the multiple-founding model over the multiple-mating model. Although we have not considered a mixture of multiple mating and multiple founding, we can conclude that multiple founding occurs in all four species. In the following analysis, we assume that all foundresses are singly mated, but that more than one such female may found a gall.

(b) *Intra- and interspecific variation in the extent of multiple founding*

Next, we ask whether there is heterogeneity between species and populations in the extent to which multiple founding occurs. This is done by allowing each species a separate parameter for the average number of females per gall. Tables 2 and 3 show the results of this analysis. We find evidence for species-level variation in the number of females per gall such that *A. coriarius* and *A. panteli* have a significantly higher ($2\Delta L = 51.24$, $p < 0.001$) average number of foundresses per gall (2.62 and 2.05, respectively) than *A. lucidus* and *A. seckendorffi* (1.36 and 1.10, respectively). The results expressed as the proportion of galls founded by a single female show a similar pattern and we present the population and species averages for the two parameters in table 2. By contrast, we find no evidence for significant intraspecific variation in foundress number, despite the wide geographical distribution of samples (table 3). The result suggests that foundress number is a characteristic of the species rather than a function of the local ecology.

dress number is a characteristic of the species rather than a function of the local ecology.

(c) *How well do the models fit the data?*

Finally, we wish to assess the goodness-of-fit between the model fitted and the data. There are several elements to consider; the most important being the assumptions of the modified Poisson distribution for the number of foundresses per gall and of the Hardy–Weinberg and linkage equilibria.

The accuracy of the modified Poisson distribution as a description of the number of foundresses per gall can be assessed by comparing the distribution of the estimated foundress number (from the maximum *a posteriori* likelihood) with that expected under the best-fitting model using a χ^2 goodness-of-fit test (figure 1). The modified Poisson distribution seems adequate in describing the estimated number of foundresses per gall for all species except *A. lucidus* ($\chi^2_1 = 4.03$, $p = 0.045$).

Although the assumptions concerning Hardy–Weinberg and linkage equilibria could be tested by the introduction of further parameters into the model, this is not of central interest here. Instead, we wish to ask whether our conclusions are robust to the assumptions we have made about oviposition behaviour. To address this issue, we can treat the mating structure that gave rise to each gall as a free parameter and find the structure (or set of equivalent structures) that maximizes the likelihood for each gall. We can then ask whether we can still detect differences between species in terms of the distribution of estimated foundress number. Figure 2 shows the comparison between the maximum *a posteriori* estimates of foundress number from the fully parameterized model, with those obtained by treating each mating structure as a separate parameter. Although treating each mating structure as a separate parameter increases the estimated average number of foundresses per gall for each species, the differences between the species remain ($\chi^2_1 = 31.22$, $p = 0.002$) and their separation into two pairs—*A. lucidus* and *A. seckendorffi* with a low rate of multiple founding, and *A. coriarius* and *A. panteli* with a high rate of multiple

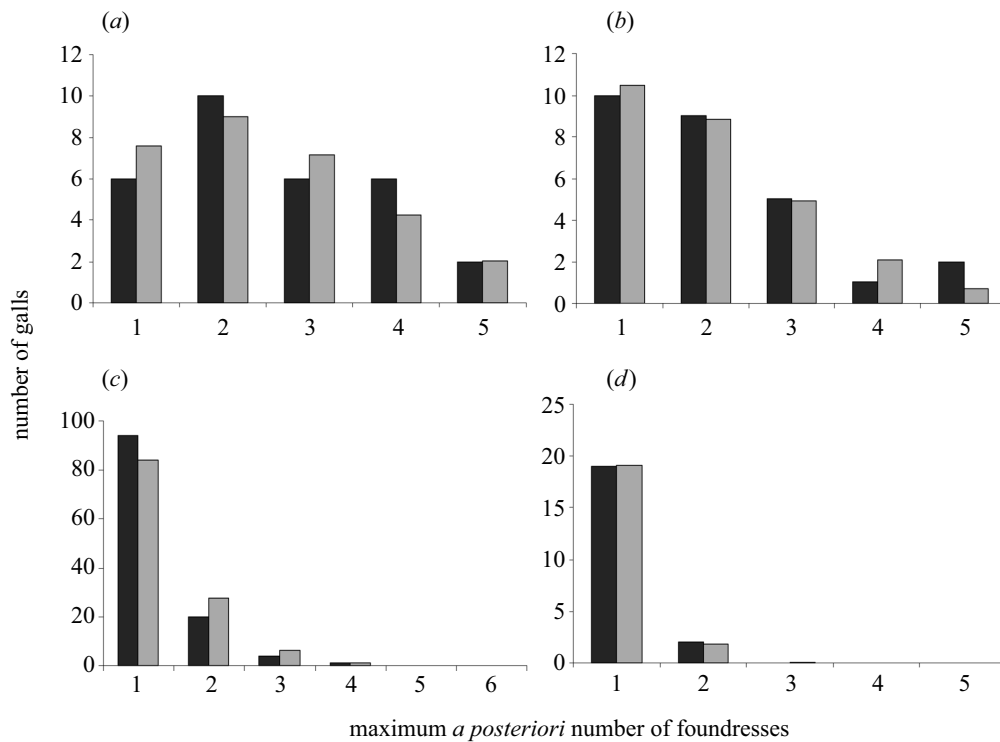


Figure 1. The distribution of maximum *a posteriori* estimates of foundress number for each species (black bars) and the expected number under the best-fitting modified Poisson distribution (grey bars). (a) *Andricus coriarius*, $\chi^2_1 = 1.09$, n.s., (b) *A. panteli*, $\chi^2_1 = 0.04$, n.s., (c) *A. lucidus*, $\chi^2_1 = 4.03$, $p = 0.045$, (d) *A. seckendorffi*, $\chi^2_1 = 0.03$, n.s.

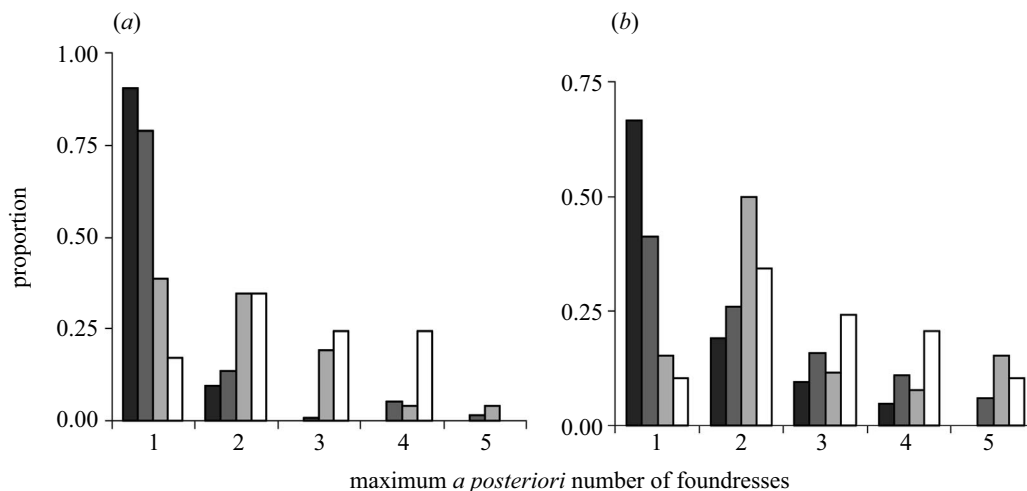


Figure 2. The distribution of estimated foundress number under (a) the fully parameterized model, and (b) when the mating structure for each gall is treated as a separate parameter. Black bars, *Andricus seckendorffi*; dark grey bars, *A. lucidus*; light grey bars, *A. panteli*; white bars, *A. coriarius*.

founding—persists. Thus, even though our model using a modified Poisson distribution assumes independence between females in their choice of oviposition site, we still find evidence for species-specific differences in multiple-founding frequency when this assumption is relaxed.

4. DISCUSSION

(a) Multiple founding

To our knowledge this is the first study demonstrating multiple founding in any insect-induced gall. It shows that the offspring of different mothers are able to coexist within

the same gall structure, and raises questions concerning the interactions among different sib groups. Do the offspring of different foundresses compete for limited resources available in the gall, or do the combined broods operate as a sink for host-plant nutrients in the same way as an equivalent number of siblings?

(b) Species-level differences in multiple founding

A striking feature of our results is that levels of multiple founding are consistent within a species, but differ among species. Even when different species have been sampled from the same location, the level of multiple founding

reflects characteristics of the species, rather than the local ecology. This suggests that foundress number is determined by geographically consistent properties of the biology of each species. A second feature is the division of the four species into two clear groups, one with a lower level of multiple founding (*A. lucidus*, *A. seckendorffi*) than the other (*A. coriarius*, *A. panteli*). An obvious question is whether the two pairings represent two phylogenetically close sets of species, each pair sharing common ancestral traits correlated to the probability of multiple founding. If this were true, we would expect *A. lucidus* and *A. seckendorffi* to be sister groups relative to a similar pairing of *A. coriarius* and *A. panteli*. However, an existing DNA sequence phylogeny of the genus *Andricus* (Stone & Cook 1998) shows that *A. panteli*, *A. lucidus* and *A. seckendorffi* are very closely related, whereas *A. coriarius* resides within a distant clade. This suggests that evolutionarily labile ecological traits underlie the two pairings.

Here, we consider three ecological hypotheses for the observed patterns of interspecific variation:

- (i) variation in population density among gallwasp species;
- (ii) variation among gallwasp species in availability of gall-induction sites; and
- (iii) variation in levels of parasitoid attack.

The first two assume no interaction among multiple foundresses, whereas the third assumes selection to co-found galls.

(i) *Variation in population density*

If the four species routinely exist at different population densities, then more abundant species would be predicted to experience higher levels of multiple founding. We have no estimates for population densities of the species involved, but it is hard to envisage a mechanism that would generate geographically consistent differences in population density.

(ii) *Availability of gall-induction sites*

Although all oak cynipids are thought to require active meristematic or otherwise totipotent cells for gall induction (Stone *et al.* 2002), it is possible that the oviposition sites required by *A. coriarius* and *A. panteli* are consistently rarer than those used by *A. lucidus* and *A. seckendorffi*, leading to higher rates of multiple founding. The four species use hosts in the same oak section (*Quercus sensu stricto*), but there are differences in the tissue type used. *Andricus panteli* and *A. coriarius* galls develop on buds, whereas *A. seckendorffi* and *A. lucidus* galls develop on acorns. However, *A. lucidus* galls are also found on buds (Nieves-Aldrey 1987; Csóka 1997; Melika *et al.* 2000), suggesting that the species-specific patterns of multiple founding may not be due solely to the relative abundance of different host tissues.

Another factor is the timing of oviposition. If this differs among species and if the density of suitable plant tissue changes over the season, females may experience different degrees of resource limitation (even when they use the same host tissue). In addition, although gall induction begins immediately after the eggs are deposited (Shorthouse & Brooks 1998) the length of time during

which other females can lay and still have their eggs incorporated into the same overall gall structure may vary between species.

Any of these factors may be important in determining species-specific levels of multiple founding, but nothing is known about the phenology of gall induction in these species as the sexual generations have yet to be identified.

(iii) *Parasitoid attack*

One possibility is that the selection pressure for multiple founding differs between species. For example, *A. coriarius* and *A. panteli* may experience uniformly higher rates of parasitoid-inflicted mortality than the other two species, leading to stronger selection for females to seek out oviposition sites that have already been used. We have no data on relative levels of parasitoid attack in the four species.

(c) *Distinguishing among alternative hypotheses*

The apparent fit between the Poisson model of foundress number and the estimated distribution of foundress number per gall suggests that multiple founding may be simply a question of resource limitation rather than active preference for sites where previous females have oviposited. The Poisson model assumes that there is a finite number of sites that can be used and that females adopt oviposition sites at random, taking no account of whether previous females have oviposited. If there was an active preference for multiple founding, we would expect the distribution to have an excess of galls with large numbers of foundresses. However, the fit of the model does not indicate that it is necessarily the correct model and it should be noted that there may not be enough power in the data analysed here to distinguish between different forms of the distribution of the number of foundresses.

The hypotheses could also be distinguished experimentally under conditions in which the density of sexual generation females could be manipulated, availability of host organs quantified and patterns of oviposition documented. The population density hypothesis predicts the proportion of host sites attacked and the probability of multiple founding to be similar for all four species at the same population density. The gall-induction site availability hypothesis predicts that interspecific differences in resource use will lead to different levels of multiple founding even if all four species occur at the same density. The parasitoid attack hypothesis predicts that the decrease in parasitoid-inflicted mortality that is associated with increase in gall size through addition of further chambers should be greatest in *A. coriarius* and *A. panteli*. It also predicts that females of these two species should show a stronger preference than *A. lucidus* and *A. seckendorffi* for buds in which conspecific females are known to have oviposited.

(d) *Wider application of the methods*

The spatial distribution of individuals and their actions are important elements in both population and evolutionary processes, but are often hard to determine. An advantage of the methods presented here is that they provide a way of detecting local population structure in nature without either direct observation or experimental manipulation.

Other methodologies have been developed that use estimates of pairwise relatedness to determine basic population structure (Queller & Goodnight 1989; Lynch & Ritland 1999). However, in contrast to our methods, these approaches require many or hypervariable loci and are not explicitly aimed at testing alternative scenarios. Examples in which our approach might complement existing work include studies of:

- (i) superparasitism by gregarious insect parasitoids (Godfray 1993);
- (ii) local mate competition and sex ratios in figwasps (Molbo & Parker 1996; West & Herre 1998); or
- (iii) mixed infections of pathogens (Hill & Babiker 1995) in a single host.

The authors thank G. Csóka, O. Plantard and S. Lourie for their help in collecting galls. R. J. A. was funded by a BBSRC studentship for this work, G. A. T. M. is funded by The Royal Society and G. N. S. is supported by grants from The Royal Society (574006) and NERC (GR/12847 and the EDGE thematic programme GR9/03553). Additional funding for field work was provided by the Henrietta Hutton Memorial Fund, the John Ray Trust and the Hope Entomological Fund.

REFERENCES

- Abrahamson, W. G. & Weis, A. E. 1997 *Evolutionary ecology across three trophic levels: goldenrods, gallmakers and natural enemies*. Princeton University Press.
- Askew, R. R. 1961 On the biology of the inhabitants of oak galls of Cynipidae (Hymenoptera) in Britain. *Trans. Soc. Br. Entomol.* **14**, 237–268.
- Askew, R. R. 1984 *The biology of gallwasps*. New Delhi: Oxford and IBH Publ.
- Atkinson, R. J. 2000 The genetic analysis of natural history, reproductive strategy and population structure in European oak gallwasps (Hymenoptera: Cynipidae). D.Phil. thesis, University of Oxford.
- Bronner, R. 1985 Anatomy of the ovipositor and oviposition behaviour of the gallwasp *Diplolepis rosae* (Hymenoptera: Cynipidae). *Can. Entomol.* **117**, 849–858.
- Charnov, E. L. & Skinner, S. W. 1984 Evolution of host selection and clutch size in parasitoid wasps. *Fl. Entomol.* **67**, 5–21.
- Craig, T. P., Itami, J. K., Shantz, C., Abrahamson, W. G., Horner, J. D. & Craig, J. V. 2000 The influence of host plant variation and intraspecific competition on oviposition preferences and offspring performance in the host races of *Eurosta solidaginis*. *Ecol. Entomol.* **25**, 7–18.
- Csóka, G. 1997 *Plant galls*. Budapest: Forest Research Institute.
- Eber, S. & Brandl, R. 1994 Ecological and genetic spatial patterns of *Urophora cardui* as evidence for population structure and biogeographical processes. *J. Anim. Ecol.* **63**, 187–199.
- Ejlertsen, A. 1978 The spatial distribution of spangle galls (*Neuroterus* spp.) on oak (Hymenoptera: Cynipidae). *Entomol. Meddel.* **46**, 19–25.
- Folliot, R. 1964 Contribution à l'étude de la biologie des cynipides gallicoles. *Ann. Sci. Nat.: Zool.* **12**, 407–564.
- Freese, G. & Zwolfer, H. 1996 The problem of optimal clutch size in a tritrophic system: the oviposition strategy of the thistle gallfly *Urophora cardui* (Diptera, Tephritidae). *Oecologia* **108**, 293–302.
- Godfray, H. C. J. 1993 *Parasitoids: behavioural and evolutionary ecology*. Princeton University Press.
- Hill, W. G. & Babiker, H. A. 1995 Estimation of numbers of malaria clones in blood samples. *Proc. R. Soc. Lond. B* **262**, 249–257.
- Jones, D. 1983 The influence of host density and gall shape on the survivorship of *Diastrophus kincaidii* Gill. (Hymenoptera: Cynipidae). *Can. J. Zool.* **61**, 2138–2142.
- Kato, K. & Hijii, N. 1993 Optimal clutch size of the chestnut gallwasp *Dryocosmus kuriphilus yasumatu* (Hymenoptera, Cynipidae). *Res. Popul. Ecol.* **35**, 1–14.
- Latto, J. & Briggs, C. J. 1995 Factors affecting distribution of the gall forming midge *Rhopalomyia californica* (Diptera, Cecidomyiidae). *Environ. Entomol.* **24**, 679–686.
- Lynch, M. & Ritland, K. 1999 Estimation of pairwise relatedness with molecular markers. *Genetics* **152**, 1753–1766.
- Melika, G., Csóka, G. & Pujade-Villar, J. 2000 Check-list of oak gall wasps of Hungary, with some taxonomic notes (Hymenoptera: Cynipidae, Cynipinae, Cynipini). *Annls Historico naturales Mus. Nationalis Hung.* **92**, 265–296.
- Molbo, D. & Parker, E. D. 1996 Mating structure and sex ratio variation in a natural population of *Nasonia vitripennis*. *Proc. R. Soc. Lond. B* **263**, 1703–1709.
- Nieves-Aldrey, J.-L. 1987 Estado actual del conocimiento de la subfamilia Cynipidae (Hymenoptera, Parasitica, Cynipidae) en la Península Ibérica. *Eos* **63**, 179–195.
- Price, P. W. & Clancy, K. M. 1986 Interactions among 3 trophic levels—gall size and parasitoid attack. *Ecology* **67**, 1593–1600.
- Queller, D. C. & Goodnight, K. F. 1989 Estimation of genetic relatedness using allozyme data. *Evolution* **43**, 258–275.
- Richardson, B. J., Baverstock, P. R. & Adams, M. 1986 *Allozyme electrophoresis*. San Diego, CA: Academic.
- Roitberg, B. D., Robertson, I. C. & Tyerman, J. C. A. 1999 Vive la variance: a functional oviposition theory for insect herbivores. *Entomol. Exp. Appl.* **91**, 187–194.
- Ronquist, F. 1999 Phylogeny, classification and evolution of the Cynipoidea. *Zool. Scripta* **28**, 139–164.
- Rossi, A. M., Stiling, P. D., Strong, D. R. & Johnson, D. M. 1992 Does gall diameter affect parasitism of *Asphondylia borrichiae* (Diptera: Cecidomyiidae)? *Ecol. Entomol.* **17**, 149–154.
- Scholz, D. & Poehling, H. M. 2000 Oviposition site selection of *Episyrphus balteatus*. *Entomol. Exp. Appl.* **94**, 149–158.
- Schönrogge, K., Harper, L. J. & Lichtenstein, C. P. 2000 The protein content of tissues in cynipid galls (Hymenoptera: Cynipidae): similarities between cynipid galls and seeds. *Plant Cell Environ.* **23**, 215–222.
- Shorthouse, J. D. & Brooks, S. E. 1998 Biology of the galler *Diplolepis rosae-folii* (Hymenoptera: Cynipidae), its associated component community and host shift to the shrub rose Theresia bugnet. *Can. J. Entomol.* **130**, 357–366.
- Stille, B. 1984 The effect of host-plant and parasitoids on the reproductive success of the parthenogenetic gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae). *Oecologia* **63**, 364–369.
- Stille, B. 1985 Population genetics of the parthenogenetic gall wasp *Diplolepis rosae* (Hymenoptera, Cynipidae). *Genetica* **67**, 145–151.
- Stone, G., Atkinson, R., Rokas, A., Csóka, G. & Nieves-Aldrey, J.-L. 2001 Differential success in northwards range expansion between ecotypes of the marble gallwasp *Andricus kollari*: a tale of two lifecycles. *Mol. Ecol.* **10**, 761–778.
- Stone, G. N. & Cook, J. M. 1998 The structure of cynipid oak galls: patterns in the evolution of an extended phenotype. *Proc. R. Soc. Lond. B* **265**, 979–988.
- Stone, G. N. & Sunnucks, P. 1993 Genetic consequences of an invasion through a patchy environment; the cynipid gallwasp *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Mol. Ecol.* **2**, 251–268.
- Stone, G. N., Schönrogge, K., Atkinson, R. J., Bellido, D. &

- Pujade-Villar, J. 2002 The population biology of oak gallwasps (Hymenoptera: Cynipidae). *A. Rev. Ecol. Syst.* **47**, 633–638.
- Weis, A. E., Price, P. W. & Lynch, M. 1983 Selective pressures on clutch size in the gall maker *Asteromyia carbonifera*. *Ecology* **64**, 688–695.
- Weis, A. E., Walton, R. & Crego, C. L. 1988 Reactive plant tissue sites and the population biology of gall makers. *A. Rev. Entomol.* **33**, 467–486.
- West, S. A. & Herre, E. A. 1998 Partial local mate competition and the sex ratio: a study on non-pollinating fig wasps. *J. Evol. Biol.* **11**, 531–548.
- Zwolfer, H. & Arnold-Rinehart, J. 1994 Parasitoids as a driving force in the evolution of gall size of *Urophora* on Carduace hosts. In *Plant galls; organisms, interactions, populations* (ed. M. A. J. Williams), pp. 245–258. Oxford: Clarendon.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.