Eur. J. Entomol. **108**: 71–78, 2011
http://www.eje.cz/scripts/viewabstract.php?abstract=1589
ISSN 1210-5759 (print), 1802-8829 (online)

Comparison of the severity of selection among beech leaves prior to egg-laying between a leaf-mining and two gall-inducing insects

JEAN BÉGUINOT

Société d'Histoire Naturelle du Creusot, 12 rue des Pyrénées, F-71200 Le Creusot, France; e-mail: jean-beguinot@orange.fr

Key words. Behaviour, oviposition, egg-laying, selectivity, host acceptance, clutch size, insect, mine, gall, inference, Lepidoptera, Gracillariidae, Diptera, Cecidomyiidae, *Mikiola fagi*, *Hartigiola annulipes*, *Phyllonorycter maestingella*

Abstract. Quantitative behavioural traits associated with egg-laying, such as the level of selectivity for host-supports and the size of egg clutches, are generally thought to be of great importance for the subsequent survival and development of offspring. These quantitative traits, however, are often difficult to assess reliably by direct observation in the field. This is particularly the case when the insects are very tiny, which is the case for most galling and leaf mining insects. However, a new approach, the "Melba" procedure, allows the indirect inference of these quantitative traits, using easily recorded field-data only. Application of this diagnostic procedure to a large series of samples of beech leaves (*Fagus silvatica*), harbouring either a leaf miner, *Phyllonorycter maestingella* (Lepidoptera: Gracillariidae) or one or the other of two galling insects, *Mikiola fagi* or *Hartigiola annulipes* (Diptera: Cecidomyiidae) indicates that the leaf miner differs significantly from the two species of galling insect in term of combined values of host-acceptance ratio and average clutch-size, while the two gall-inducing species remain substantially undistinguishable from each other according to these traits. Thus, the galling insects (i) show stronger selectivity for a host than does the miner at any given average clutch-size and (ii) show larger average clutch-size at any given level of selectivity. That is, for at least these three species, the galling insects show a greater level of selectivity when choosing leaves to oviposit on but, then, tend to lay larger egg-clutches. These differences may be due (i) to the gall-inducing process requiring far more of leaf tissues than being simply palatable, which makes it likely that galling species will be more selective in their choice of leaves than leaf miners and (ii) to the capacity of galls to become nutrient sinks, which may help explain why the galling insects laid larger egg clutches. However, whether these trends can be regarded as general rather specific to this particular case, depends on the outcome of future studies on other groups of insects with similar life histories.

INTRODUCTION

Optimising the survival and future success of offspring in insects arguably implies that mothers lay eggs where there are the appropriate resources for the development of their larvae (Vuorisalo et al., 1989; Thompson & Pellmyr, 1991; Réale & Roff, 1992; Awmack & Leather, 2002; Fox & Czesak, 2002; Obermaier et al., 2008; Fry et al., 2009; Morrison & Quiring, 2009; Trager et al., 2009; although sometimes it is difficult to determine a clear relationship: Gripenberg et al., 2007b). The likely reason for this is that, usually, the larval stage is both the least mobile and the one mainly devoted to feeding in insects. Accordingly, the location of larval development, if it depends upon the place where eggs were laid, is likely to affect larval growth and the resulting insect fitness. Even the more "classical" herbivores (free-feeders such as chewers or more "subtle" borers and skeletonizers) have more or less particular food requirements, with many being to some extent oligotrophous or even (sub-) monophagous (Jolivet, 1983; Carrière, 1998). This, evidently, is especially the case when the larval stage must remain within a host or part of a host, such as (endo-) parasitoids and galling or mining insects. In galling and mining insects the larvae complete their development often within one discrete, clearly-bounded host-part, hereafter designated a "host-unit". This may be a leaf, bud, flower, fruit or particular section of shoot of a given host-species (equivalent to individual host of similar age, belonging to the same species, in parasitoid insects).

In quantitative terms, the degree of selectivity of egg-laying mothers among available host-units may be quantified by the acceptance ratio "α" , i.e. the proportion of visited host-units that are accepted for egg-laying by a gravid mother. The notion that insects select from among the host-units available, quantified in terms of the acceptance ratio, makes sense because the host-units, for example, the leaves of a given plant or tree, are generally of unequal "quality" regarding future larval development (resources, protection, etc.). Thus, the geometrical, physical, chemical and other characteristics of the same type of host-unit may often vary in space and time (Morrison & Quiring, 2009; Sarfraz et al., 2009; Zehnder et al., 2009), for example, leaves may vary in quite an unsuspected manner in many traits within the canopy of an individual tree or even within the same branch (Faeth, 1991; Kozlov & Koricheva, 1991; Gripenberg, 2007). At least, some of these characteristics may affect the development of the larvae growing upon or within a host-unit. Apparently, gravid females are able to discriminate according to these characters and select opportunely between adjacent host-units (Vuorisalo et al., 1989; Heimpel & Rosenheim, 1995; Cope & Fox, 2003; Gibbs et al., 2005; Gripenberg & Roslin, 2005; Roslin et al., 2006; Gripenberg et al., 2007a, b; Sarfraz et al., 2009; Trager et al., 2009). While such variations in quality among units on a given host-plant are usually gradual (Dajoz, 1993), females, however, are compelled to a two-fold "go – no go" response, either accepting or not accepting a given unit for subsequent egg-laying.

Then, after having selected an appropriate unit, another decision, of no less importance for future larval development, is the number of eggs a gravid female will deposit during its visit to this unit. The average number of eggs, deposited as a single bout, during a visit to an acceptable host-unit, is commonly referred to as the "clutch size" (hereafter designed as "n_c").

For leaf miners and galling insects, selectivity for host-part quality is likely to be more important for the latter, since for them the selection of a suitable host-unit for oviposition needs to take into account not only its future quality in terms of a source of food but also, and decisively, whether it can be induced by the insect to produce a suitable gall. Accordingly, the relationships between the insect and its host are likely to be even more interconnected for galling insects than leaf miners. Note that whether the mother induces the development of the gall or the hatching larva itself (as in Cecidomyiidae) is immaterial to the fact that the choice of suitable host unit is made by the mother prior to egg-laying.

Comparative studies of leaf miners and galling insects are best done on species exploiting the same type of host-unit on the same host-species. This, indeed, is not difficult since most gall-supporting plant-species may also harbour mining insects in the same host-units. On the other hand, the results and trends derived from this study may not be general but specific to the species studied.

In the present study, the selectivity of two galling insects and one leaf miner exploiting the same host-units on the same species of host, the leaves of beech-trees *Fagus sylvatica*, were compared. The gall-inducing species were *Mikiola fagi* (Hartig) and *Hartigiola annulipes* (Hartig) (Diptera: Cecidomyiidae) and the leaf mining species *Phyllonorycter maestingella* (Müller) (Lepidoptera: Gracillariidae). All three species are common and consequently, large censuses may be conducted in the field at many locations, which is desirable from a statistical point of view.

The following questions regarding these three common herbivore-insect species feeding on beech leaves are addressed:

(1) Do the two gall-inducers substantially differ from the leaf miner in terms of quantitative aspects of their behaviour prior to and during oviposition, that is, do the gall-inducers and leaf miner show significantly different ranges of values for either (or both) α, the "leaf acceptance ratio" and n_c, the average "clutch size"?

(2) For each of the three species studied, how large is the range in variability of the leaf acceptance ratio α, a question that may be connected to differences in the selective behaviour of the mothers and also to the degree of the between tree variability in terms of average leaf quality.

METHODS

Although the "acceptability" or "non acceptability" of host-units, in this case leaves, by ovipositing mothers makes sense, estimating the proportion of acceptable units (the acceptance ratio α) is far from straightforward in practice. While the number of actually accepted leaves is easy to determine, they form just a part of the set of potentially acceptable leaves, a part which is dependent upon the density of ovipositing insects. Therefore, the proportion of leaves accepted is a poor predicator, an undefined underestimate of the proportion of potentially acceptable leaves. It is the proportion of the potentially acceptable leaves (not those actually accepted) that is meaningful in terms of mothers' requirements. Consequently, the proportion of the leaves accepted may definitely not serve as a surrogate for determining the leaf-acceptance ratio α. The same holds true for the total number of eggs laid on a given leaf, an easily recorded but evidently poor predicator of clutch size n_c, since several batches of eggs may be successively laid on the same leaf.

Of course, good estimates of α and n_c can be obtained from well defined, tedious experimental protocols (see for example Gripenberg, 2007). Alternatively, far easier and rapid determinations of α and n_c may be obtained indirectly by using an appropriate model implemented with easily recorded field census data. Accordingly, this second approach is especially interesting when it is planned to analyze numerous sets of data. Indeed, such extensive surveys, involving many different samples from different locations are now considered of fundamental importance to ensure a comprehensive grasp of plant-animal relationships. This is because contextual variability may substantially influence and appreciably modulate more deterministic ecological relationships (Trager et al., 2009; Zehnder et al., 2009). As quoted by Gripenberg & Roslin (2007), "the mere spatial location of a site will have major impact on local insect populations", and thus, "descriptive studies of individual habitats or sites will fall short of generality".

Thus, an indirect but more rapid procedure for determining α and n_c, namely the "Melba" procedure was used (for a detailed description of the procedure see Béguinot, 2005, 2009a, b and the Appendix hereafter for the particular treatment of the average clutch-size). This procedure relates the unknown parameters α and n_c to the easily recorded distribution $\Pi(\eta)$ of the total number η of eggs laid per leaf (or the resulting artefacts such as mines or galls) all over the entire set of leaves within the sample. Briefly, the procedure is based on the fact that, thanks to acceptable simplifying assumptions, the distribution $\Pi(\eta)$ should theoretically be of a specific type with α and n_c as the adaptable parameters. Conversely, the inferred values for α and n_c are those that result in the best fit between the recorded distribution $\Pi(\eta)$ and the corresponding theoretical distributions $\Pi(\eta)$, parameterised in terms of α and n_c. Here, the field data is the distribution of singly-occupied galls or mines (which reflects that of those eggs that have successfully hatched), rather than the distribution of eggs, since here, the former is more easily recorded. Accordingly, it should be remembered that, hereafter, the term "clutch-size" n_c will apply exclusively to the number of artefacts (galls or mines) that resulted from one clutch of eggs; the "artefact-clutch" size thus corresponds to the "eggs-clutch" size reduced in the proportion of eggs that have successfully hatch [the "Melba" procedure remains equally appropriate in this case as it is independent of the proportion of eggs that successfully hatch: see Béguinot (2005, 2009a, b)].

Field data used in this study (Table 1) include a substantial series of 66 samples, each one from a different tree, collected in the central part of France: Burgundy, Limousin and Hautes-

Table 1. Leaf-acceptance ratio, α, and average clutch size, n_c, (= number of artefacts resulting from one egg-clutch), for leaf gallers (*Hartigiola annulipes*, *Mikiola fagi*) and a leaf-miner (*Phyllonorycter maestingella*) based on a series of samples of leaves collected from *Fagus silvatica.* Contextual parameters: n_u = number of leaves in the sample, n_a = total number of galls or mines recorded in the sample, n_{fa} = number of leaves with mines or galls in the sample. Oviposition pressure: $n_a/(\alpha n_u)$, mines/galls density: n_a/n_u.

Locality / reference	Date	Insect	n_u	n_a	n_{fa}	α	n_c	Overall density of mines/galls	Oviposition pressure
Bourgogne	28.viii.2005	*Hartigiola*	565	253	167	0.72	1.09	0.45	0.62
Bourgogne	28.viii.2005	*Hartigiola*	761	73	52	0.19	1.07	0.10	0.50
Bourgogne	28.viii.2005	*Hartigiola*	232	388	105	0.58	1.33	1.67	2.88
Bourgogne	28.viii.2005	*Hartigiola*	468	58	44	0.33	1.05	0.12	0.38
Bourgogne	2.x.2005	*Hartigiola*	342	140	62	0.33	1.38	0.41	1.24
Bourgogne	2.x.2005	*Hartigiola*	391	172	73	0.29	1.20	0.44	1.52
Bourgogne	11.x.2005	*Hartigiola*	733	485	255	0.63	1.14	0.66	1.05
Bourgogne	14.x.2005	*Hartigiola*	280	162	80	0.45	1.17	0.58	1.29
Bourgogne	26.x.2005	*Hartigiola*	454	69	43	0.22	1.10	0.15	0.69
Bourgogne	23.ix.2008	*Hartigiola*	354	355	162	0.73	1.21	1.00	1.37
Bourgogne	12.x.2008	*Hartigiola*	708	1243	330	0.60	1.37	1.76	2.93
Bourgogne	29.ix.2008	*Hartigiola*	404	80	52	0.33	1.09	0.20	0.60
Dajoz		*Hartigiola*	1484	697	281	0.30	1.20	0.47	1.57
Hautes-Alpes	6.viii.2005	*Hartigiola*	1700	290	110	0.09	1.24	0.17	1.90
Hautes-Alpes	6.viii.2005	*Hartigiola*	1400	129	48	0.05	1.28	0.09	1.84
Limousin	20.viii.2005	*Hartigiola*	140	13	7	0.07	1.15	0.09	1.33
Limousin	5.xi.2008	*Hartigiola*	394	691	221	0.73	1.30	1.75	2.40
Bourgogne	10.vii.2009	*Mikiola*	207	21	16	0.22	1.06	0.10	0.46
Bourgogne	10.vii.2009	*Mikiola*	671	110	73	0.18	1.00	0.16	0.91
Bourgogne	10.vii.2009	*Mikiola*	534	47	37	0.22	1.05	0.09	0.40
Bourgogne	3.ix.2009	*Mikiola*	211	78	43	0.40	1.13	0.37	0.92
Bourgogne	3.ix.2009	*Mikiola*	175	38	24	0.27	1.11	0.22	0.80
Bourgogne	3.ix.2009	*Mikiola*	455	67	50	0.30	1.07	0.15	0.49
Bourgogne	7.x.2008	*Mikiola*	561	165	94	0.34	1.16	0.29	0.87
Bourgogne	7.x.2008	*Mikiola*	790	87	58	0.21	1.19	0.11	0.52
Bourgogne	7.x.2008	*Mikiola*	559	20	14	0.05	1.06	0.04	0.72
Bourgogne	7.x.2008	*Mikiola*	585	27	24	0.18	1.00	0.05	0.26
Bourgogne	29.ix.2008	*Mikiola*	404	45	36	0.32	1.05	0.11	0.35
Bourgogne	6.v.2009	*Mikiola*	618	110	80	0.34	1.07	0.18	0.52
Bourgogne	28.v.2009	*Mikiola*	618	172	106	0.27	1.00	0.28	1.03
Bourgogne	29.v.2009	*Mikiola*	2488	1133	662	0.51	1.12	0.46	0.89
Dajoz		*Mikiola*	1484	240	142	0.19	1.12	0.16	0.85
Hautes-Alpes	6.viii.2005	*Mikiola*	1700	220	140	0.17	1.10	0.13	0.76
Hautes-Alpes	6.viii.2005	*Mikiola*	2500	432	275	0.24	1.12	0.17	0.72
Limousin	5.xi.2008	*Mikiola*	394	57	45	0.39	1.05	0.14	0.37
Limousin	7.viii.2009	*Mikiola*	365	27	22	0.23	1.05	0.07	0.32
Limousin	8.viii.2009	*Mikiola*	202	43	29	0.40	1.08	0.21	0.53
Limousin	8.viii.2009	*Mikiola*	163	17	11	0.10	1.00	0.10	1.04
Limousin	8.viii.2009	*Mikiola*	541	43	33	0.18	1.06	0.08	0.44
Limousin	8.viii.2009	*Mikiola*	563	66	50	0.25	1.07	0.12	0.47
Limousin	10.viii.2009	*Mikiola*	351	51	29	0.12	1.02	0.15	1.21
Limousin	23.viii.2009	*Mikiola*	1465	157	99	0.14	1.12	0.11	0.77
Limousin	24.viii.2009	*Mikiola*	479	72	54	0.34	1.09	0.15	0.44
Limousin	26.viii.2009	*Mikiola*	358	155	105	0.66	1.10	0.43	0.66
Bourgogne	23.viii.2005	*Phyllon.*	190	50	42	0.67	1.00	0.26	0.39
Bourgogne	23.viii.2005	*Phyllon.*	241	108	81	0.90	1.07	0.45	0.50
Bourgogne	5.ix.2005	*Phyllon.*	812	285	225	0.96	1.06	0.35	0.37
Bourgogne	3.ix.2005	*Phyllon.*	964	183	158	0.82	1.03	0.19	0.23
Bourgogne	3.ix.2005	*Phyllon.*	1009	231	193	0.84	1.04	0.23	0.27
Bourgogne	27.ix.2005	*Phyllon.*	352	88	62	0.40	1.08	0.25	0.63
Bourgogne	19.x.2005	*Phyllon.*	577	113	96	0.75	1.04	0.20	0.26
Bourgogne	2.x.2005	*Phyllon.*	342	44	38	0.40	1.00	0.13	0.32
Bourgogne	2.x.2005	*Phyllon.*	391	45	41	0.58	1.00	0.12	0.20
Bourgogne	11.x.2005	*Phyllon.*	733	371	270	0.82	1.04	0.51	0.62
Bourgogne	14.x.2005	*Phyllon.*	280	32	29	0.54	1.00	0.11	0.21
Bourgogne	14.x.2005	*Phyllon.*	344	107	90	0.97	1.02	0.31	0.32
Bourgogne	14.x.2005	*Phyllon.*	421	119	100	0.82	1.01	0.28	0.34
Bourgogne	26.x.2005	*Phyllon.*	454	152	124	0.75	1.00	0.33	0.45
Bourgogne	26.x.2005	*Phyllon.*	305	267	169	1.00	1.05	0.88	0.88
Bourgogne	26.x.2005	*Phyllon.*	206	158	108	1.00	1.00	0.77	0.77
Bourgogne	11.xi.2005	*Phyllon.*	498	59	53	0.51	1.00	0.12	0.23
Bourgogne	23.ix.2008	*Phyllon.*	354	64	53	0.64	1.05	0.18	0.28
Bourgogne	29.ix.2008	*Phyllon.*	404	55	51	0.85	1.00	0.14	0.16
Limousin	29.x.2005	*Phyllon.*	368	55	48	0.50	1.00	0.15	0.30
Limousin	30.x.2005	*Phyllon.*	396	172	63	0.62	1.00	0.18	0.29
Limousin	12.viii.2009	*Phyllon.*	505	68	64	1.00	1.00	0.13	0.13

Alpes, during the years 2005 to 2009, for each of the three species considered (Béguinot, unpubl. data). Two supplementary samples from Dajoz (1981) comply well with our more recent samples and were also included in this study. In total, more than 10,000 mines or galls were recorded on the ca. 36,000 leaves of *Fagus silvatica* surveyed. Individual samples usually consisted of all the leaves collected from one or several adjacent branches (as a rule however, leaves of the second aestival flush were duly not included in the census).

RESULTS

Sixty-six samples (respectively 17, 27, 22 samples for *Hartigiola annulipes*, *Mikiola fagi* and *Phyllonorycter maestingella*) were successively analyzed using the Melba procedure in order to quantitatively evaluate the following behavioural traits: the leaf-acceptance ratio α and the average clutch size n_c in term of the mines/galls that resulted from the eggs that successfully hatched.

Combined distributions of the behavioural traits α and n_c

Considering the results for the three species taken altogether, the leaf-acceptance ratio α extends homogeneously across almost its entire possible range, from 0.05 to 1.00 and the average clutch size, n_c, ranges from 1.00 to 1.38. Considering the species separately however, indicates that *Phyllonorycter maestingella* differs from the two other species, *Hartigiola annulipes* and *Mikiola fagi*, in terms of the ranges of values of its behavioural traits. The trend, however, is weak, with large overlaps when the traits α and n_c are considered separately, but is clear when combined values of α and n_c are considered, which reveals that the respective domains of the galling insects *Hartigiola* and *Mikiola* on the one hand and the leaf miner *Phyllonorycter* on the other hand are strikingly distinct from each other (Fig. 1). The domain of the combined values $\{\alpha, n_c\}$ for the leaf miner *Phyllonorycter* is confined, with only one exception out of 22, within a small triangle of comparatively high host acceptance ratios and low average clutch sizes and thus clearly separate from the common domain of *Hartigiola* and *Mikiola* (Chi^2 test for separation between the domains of combined values $\{\alpha, n_c\}$ for galling insects and leaf miner respectively: $\chi^2 = 57$ (with Yates correction), $p < 0.001$). This trend in the mutually exclusive domains of the combined values $\{\alpha, n_c\}$, depending on species identity (or guild membership), is all the more interesting because these behavioural traits, especially the leaf acceptance ratio, is very variable intra-specifically.

Variability in the leaf acceptance ratio within insect species

Even within a species there is a large variability in the leaf acceptance ratio α (Fig. 1). If this variability would mainly reflect large differences in leaf quality requirements of individual conspecific insects, it would be surprising because it is an important behavioural trait. Therefore, variation in other factors that could affect the leaf acceptance ratio needs to be checked. Thus, the variability in the local population density of insects (and the resulting oviposition pressure) and the variability in average leaf quality between samples (i.e. between individual trees) were also considered.

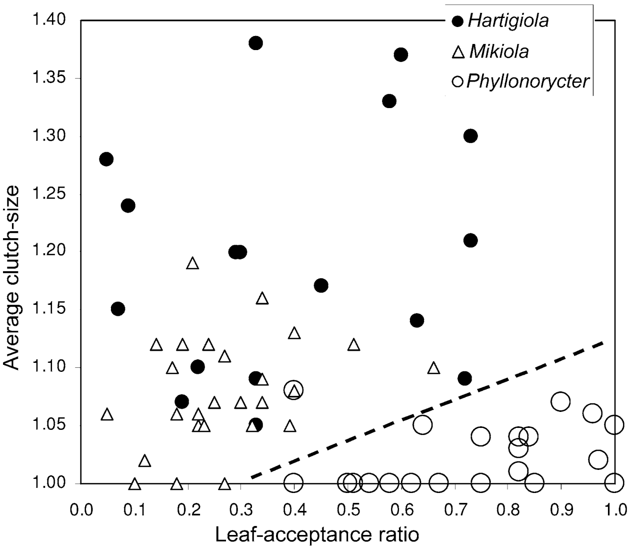

Fig. 1. Leaf acceptance ratio, α, and average clutch size, n_c, of *Hartigiola annulipes*, *Mikiola fagi* (galling insects) and *Phyllonorycter maestingella* (leaf miner).

Figs 2 and 3 provide information relevant to these suggestions.

There is no correlation between the leaf acceptance ratio, α, and the number of mines or galls per potentially acceptable leaf, which is used here as a measure of oviposition pressure: coefficient of determination $r^2 = 0.06$, 0.03, 0.07 for *Hartigiola*, *Mikiola* and *Phyllonorycter* respectively (Fig. 2). On the other hand, the leaf acceptance ratio, α, has a close association with the overall density of mines/galls, δ (number of mines or galls per leaf, considering all the leaves in the sample whether they were potentially acceptable or not): $r^2 = 0.72$, 0.65, 0.38 for *Hartigiola*, *Mikiola* and *Phyllonorycter*, respectively (Fig. 3). As will be discussed further later on, this linkage between α and δ could actually reflect their common dependence upon between-tree heterogeneity in term of average leaf quality and thus support the likely influence of between-tree heterogeneity upon the large variability of the acceptance-ratio α within a same insect species.

DISCUSSION

Combined distributions of the oviposition behavioural traits α and n_c

The distributions of the leaf acceptance ratio, α, and the average clutch size, n_c, (Fig. 1) indicate significantly distinct patterns for *Hartigiola annulipes* and *Mikiola fagi*, the two gall insects, and *Phyllonorycter maestingella*, the leaf miner, although all three species feed on beech leaves:

- the two galling insects show stronger levels of selectivity for host quality than the leaf miner, at any given value of average clutch size;

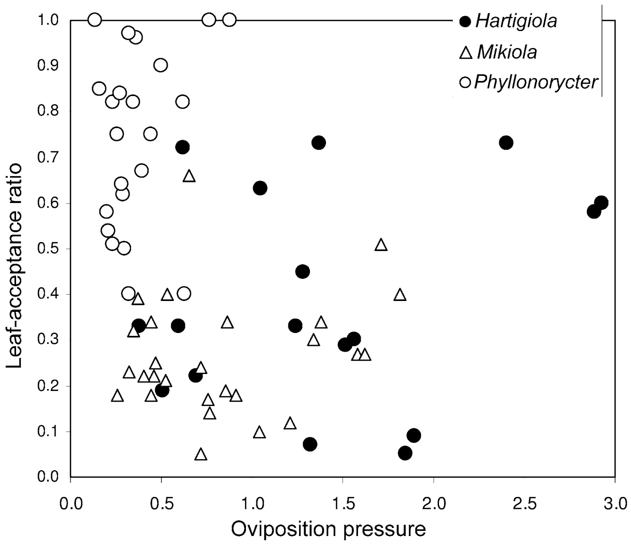

Fig. 2. Leaf acceptance ratio, α, plotted against oviposition pressure, defined as the number of mines/galls per potentially acceptable leaf.

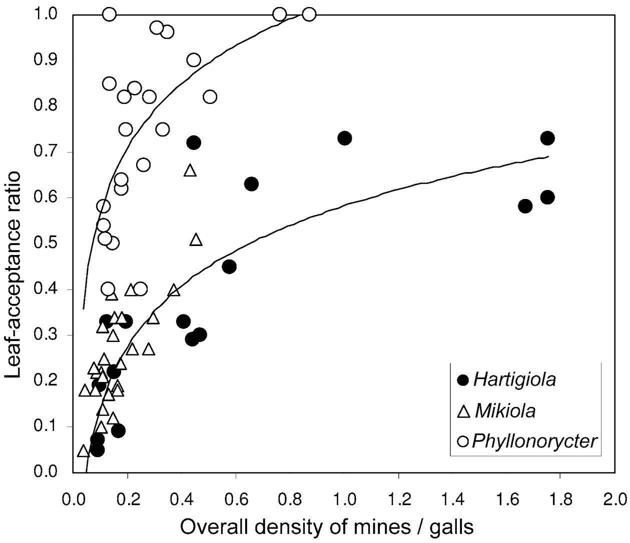

Fig. 3. Leaf acceptance ratio, α, plotted against the overall density of mines/galls in the sample, δ.

- and, reciprocally, the two galling insects have a larger average clutch size than the leaf miner, at any given level of leaf selectivity.

In short, at least for these three species, the two galling insects are clearly more selective when choosing leaves on which to oviposit and lay more eggs per visit than the leaf miner. Strong selection for high host-plant quality in galling insects is previously reported by Craig et al. (1986), Burnstein & Wool (1993, see also references therein), Stein & Price (1995), Fernandes et al. (2000), but see also Larsson & Ekbom (1995).

The ability of mothers to select among leaves prior to egg laying is all the more likely given that the variability in leaf quality seems generally to be even greater within than between trees (Suomela & Ayres, 1994; Wallin & Raffa, 1998; Gripenberg & Roslin, 2005; Roslin et al., 2006; Gripenberg et al., 2007a, b). Therefore, a substantial part of the whole range in leaf quality occurs within a limited area, thus making it easy for insects to "shop" for those leaves that are the best for their needs. Selection for quality is commonly reported in insects (Vuorisalo et al., 1989; Gripenberg & Roslin, 2007; Gripenberg et al., 2007a). It is a logical, although not always experimentally supported assumption (Mayhew, 2001), that this selection of a high quality host prior to egg-laying is associated with improved fitness of their offspring, especially when the latter are more or less dependent on one host-unit only for their larval development (see Mayhew, 1997).

Note that as the resulting galls/mines were recorded here instead of eggs, it might be argued that the clumped distribution of galls or mines could proceed a posteriori from a difference in the ratio of eggs to mines/galls depending on leaf characteristics, rather than the consequence of the selection of oviposition sites by the insects a priori. In both cases however, it is easily verified that the expression and estimate of the acceptance ratio, α, would remain strictly unchanged (Béguinot, 2009a, b). Actually, while the selection by insects based on host quality has occasionally been challenged (Burnstein & Wool, 1993), it is generally accepted, particularly for gall inducing insects, as mentioned above.

As it is generally accepted that insects select where they lay their eggs it should prove revealing to proceed in further comparisons regarding the levels of host-quality selection either within insect species in which the populations are differently structured and located in different places, or between species and especially between groups of species, such as free-feeders, leaf miners or galling insects, which are likely to have substantially different requirements.

This would entail very large investigations, involving both extensive field surveys of large sets of insect species and a wide range in contextual variation of the hosts (Berenbaum, 1981; Brewer & Gaston, 2003). Studying such a wide and comprehensive framework requires a convenient and rapid method for evaluating host acceptance, such as the "Melba" procedure.

Variability in the leaf acceptance ratio within insect species

As already suggested, the large variability in the degree of selectivity shown by a host-specific species (Fig. 1) seems, at first sight, somewhat surprising for a specific behavioural trait that is likely to have large affect on insect fitness. But variations in the leaf acceptance ratio do not necessarily indicate a similar variation in the insect's requirement for leaf quality but may result from variations in environmental conditions as well. Among the environmental factors listed are (i) the influence of oviposition pressure, which may induce females to be less choosy about leaf quality in order to avoid overcrowding and (ii) the influence of the average leaf quality of each individual host, which may mechanically determine the proportion, α, of acceptable leaves, i.e. the proportion of leaves that are, at least, at the supposedly constant threshold level of quality required by the females.

Oviposition pressure may be measured by the density of mines/galls on either all the leaves in a sample or only those that are potentially acceptable. As the host leaves on which the eggs are laid are by definition potentially acceptable leaves, the oviposition pressure should be measured in terms of the potentially acceptable leaves. Fig. 2 shows that, in spite of the appreciably large range of its variation within each species, the leaf acceptance ratio, α, remains substantially independent of oviposition pressure so defined. As a consequence, oviposition pressure is not likely to play a significant role in the large intra-specific variability in the acceptance ratio, α. Thus, none of the three species studied seems to be sensitive to oviposition pressure, i.e. they do not avoid ovipositing on leaves on which there are already eggs from conspecifics. While this apparent lack of sensitivity or, at least, lack of reaction of females to the presence of previously deposited eggs, is far from general in insects, it is frequently reported for small insects such as galling insects and leaf miners (Auerbach & Simberloff, 1989; Kagata & Ohgushi, 2002; Cornelissen & Stiling, 2006) and was especially clearly demonstrated experimentally for a common and frequently studied leaf miner on *Quercus* leaves, *Tischeria ekebladella* (Gripenberg, 2007).

According to Fig. 3, the leaf acceptation ratio, α, significantly increases with the overall density of mines/galls, δ, and there is a similar trend in the results for each of the three species studied.

This positive relationship is not likely to result from any direct correlation between α and insect density since the leaf acceptance ratio, α, is, as indicated above, independent of oviposition pressure. Rather it is determined by the positive dependence of both α and δ (the latter related to the population density of females) on the global level of "quality" of the tree from which the sample was collected. The better the "quality" of the tree, (i) the higher the acceptance ratio, α, will be (providing the requirement threshold remains unchanged) and, (ii) the higher the number of females attracted to a tree or the longer they will remain there, the higher δ will be. Parameters α and δ would then be indirectly connected in this way. It is then suggested that the variability in average leaf quality between trees, or even between leaves on branches of the same tree, may be important in determining the large range in intra-specific variability in the leaf acceptance ratio.

Of course, the results and trends derived above are, a priori, significant only for the host-herbivore system studied. Although complying with the logical arguments put forward previously, the difference in the trends recorded for the two galling insects and the leaf miner feeding on beech leaves have to be compared with that of insects feeding on other host species before any generalisation may be tentatively proposed. Indeed, it is anticipated that results might differ for other herbivores feeding on other species of hosts. For example, the significantly higher content of deterrent substances in some highly defended plants might result in leaf miners becoming as selective for leaf acceptability as gall inducing species feeding on the same host. This, indeed, would remain in keep with the preceding logic.

ACKNOWLEDGEMENTS. To an anonymous reviewer for significant help improving the English.

REFERENCES

AUERBACH M. & SIMBERLOFF D. 1989: Oviposition site preference and larval mortality in a leaf-mining moth. *Ecol. Entomol.* **14**: 131–140.

AWMACK C.S. & LEATHER S.R. 2002: Host-plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* **47**: 817–844.

BÉGUINOT J. 2005: Comparing the mothers selectivity for leaf quality prior to oviposition in two galling insects (Hymenoptera: Cynipidae), using a new methodological approach for behaviour analysis. *Mém. Soc. Hist. Nat. Creusot* **1**: 1–14.

BÉGUINOT J. 2008: Quantitative assessment of egg-laying behavioural parameters in insects: refining the original "Melba" procedure to cope with non-integer values of the average clutch-size. *Mém. Soc. Hist. Nat. Creusot* **4**: 37–46.

BÉGUINOT J. 2009a: The remarkably low leaf-selectivity prior to oviposition in the moth-pest Cameraria ohridella is not unique to this species within the genus Cameraria. *Rev. Ecol. (Terre & Vie)* **64**: 333–341.

BÉGUINOT J. 2009b: Comparing the ratios of leaf-acceptance prior to egg-laying among several species of leaf-mining moths upon Quercus sp. pl. (Lepidoptera: Gracillariidae, Tischeriidae). *Entomol. Gener.* **32**: 145–154.

BERENBAUM M. 1981: Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae-plant chemistry and community structure. *Ecology* **62**: 1254–1266.

BREWER A.M. & GASTON K.J. 2003: The geographical range structure of the holly leaf-miner. II. Demographic rates. *J. Anim. Ecol.* **72**: 82–93.

BURNSTEIN & WOOL D. 1993: Gall aphids do not select optimal galling sites (Smynthurodes betae; Pemphigidae). *Ecol. Entomol.* **18**: 155–164.

CARRIERE Y. 1998: Constraints on the evolution of host choice by phytophagous insects. *Oikos* **82**: 401–406.

COPE J.M. & FOX J.W. 2003: Oviposition decision in the seed beetle Callosobruchus maculatus (Coleoptera: Bruchidae): effects of seed size on superparasitism. *J. Stor. Prod. Res.* **39**: 355–365.

CORNELISSEN T. & STILING P. 2006: Clumped distribution of oak leaf miners between and within plants. *Basic Appl. Ecol.* **9**: 67–77.

CRAIG T.P., ITAMI J.K. & PRICE P.W. 1986: Resource regulation by a stem-galling sawfly on the arroyo willow. *Ecology* **67**: 419–425.

DAJOZ R. 1981: Lois de distribution de galles de diptères sur les feuilles du Hêtre. *Cah. Nat. Bull. N.P. (n.s.)* **37**: 75–79.

DAJOZ R. 1993: Quelques aspects des relations plantes-insectes; leur importance dans la dynamique des populations d'insectes phytophages. *Cah. Nat. Bull N.P. (n.s.)* **48**: 87–103.

FAETH S.H. 1991: Novel aspects of host tree resistance to leaf miners. In Baranchikov Y.N., Mattson W.J., Hain F.P. & Payne T.L.: *Forest Insects Guilds: Patterns of Interaction with Host Trees*. U.S.D.A. Forest Service, Gen. Tech. Rep. NE-153, pp. 219–239.

FERNANDES G.W., PRICE P.W., GONCALVES-ALVIM S.J., CRAIG T.P. & YANEGA D. 2000: Response of the galling insect Aciurina trixa Curran (Diptera: Tephritidae) to host plant quality. *Ann. Soc. Entomol. Brasil* **29**: 423–431.

Fox C.W. & Czesak M.E. 2002: Evolutionary ecology of progeny size in arthropods. *Annu. Rev. Entomol.* **45**: 341–369.

Fry H.R.C., Quiring D.T., Ryall K.L. & Dixon P.L. 2009: Influence of intra-tree variation in phenology and oviposition site on the distribution and performance of Ennomos subsignaria on mature sycamore maple. *Ecol. Entomol.* **34**: 394–405.

Gibbs M., Lace L.A., Jones M.J. & Moore A.J. 2005: Egg size-number trade-off and a decline in oviposition site choice quality: female Pararge aegeria butterflies pay a cost of having males present at oviposition. *J. Insect Sci.* **5**: 39–48.

Gripenberg S. 2007: *Spatial Ecology of a Specialist Insect Herbivore – the Leaf-mining Moth Tischeria ekebladella on the Pedunculate Oak Quercus robur*. Thesis, University of Helsinki.

Gripenberg S. & Roslin T. 2005: Host plants as islands: resource quality and spatial setting as determinant of insect distribution. *Ann. Zool. Fenn.* **42**: 335–345.

Gripenberg S. & Roslin T. 2007: Up or down in space? Uniting the bottom-up and top-down paradigm and spatial ecology. *Oikos* **116**: 181–188.

Gripenberg S., Salminen J.P. & Roslin T. 2007a: A tree in the eyes of a moth – temporal variation in oak leaf quality and leaf-miner performance. *Oikos* **116**: 592–600.

Gripenberg S., Morrien E., Cudmore A., Salminen J.P. & Roslin T. 2007b: Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *J. Anim. Ecol.* **76**: 854–865.

Heimpel G.E. & Rosenheim J.A. 1995: Dynamic host feeding by the parasitoid Aphytis melinus: the balance between current and future reproduction. *J. Anim. Ecol.* **64**: 153–167.

Jolivet P. 1983: Insectes et plantes: Evolution parallele et adaptation. *Bull. Soc. Linn. Lyon (Suppl.)* **52**: 164 pp.

Kagata H. & Ohgushi T. 2002: Effects of multiple oviposition on clutch size in a leaf-mining moth, Paraleucoptera sinuella (Lepidoptera: Lyonetiidae). *Entomol. Sci.* **5**: 407–410.

Kozlov M.V. & Koricheva Y.G. 1991: The within-tree distribution of caterpillar mines. In Baranchikov Y.N., Mattson W.J., Hain F.P. & Payne T.L.: *Forest Insects Guilds: Patterns of Interaction with Host Trees*. U.S.D.A. Forest Service, Gen. Tech. Rep. NE-153, pp. 240–255.

Larrson S. & Ekbom B. 1995: Oviposition mistakes in herbivorous insects: confusion or a step towards new host plant? *Oikos* **72**: 155–160.

Mayhew P.J. 1997: Adaptative patterns of host-plant selection by phytophagous insects. *Oikos* **79**: 417–428.

Mayhew P.J. 2001: Herbivore host choice and optimal bad motherhood. *Trends Ecol. Evol.* **16**: 165–167.

Morrison A. & Quiring D. 2009: Intra-plant heterogeneity influences the preference and performance of juveniles and adults of a gall midge. *Ecol. Entomol.* **34**: 287–295.

Obermaier E., Heisswolf A., Poethke H.J., Randlkofer B. & Meiners T. 2008: Plant architecture and vegetation structure: two ways for insect herbivores to escape parasitism. *Eur. J. Entomol.* **105**: 233–240.

Réale D. & Roff D.A. 1992: Quantitative genetics of oviposition behaviour and interactions among oviposition traits in the sand cricket. *Anim. Behav.* **64**: 397–406.

Roslin T., Gripenberg S., Salminen J.P., Karonen M., O'Hara R.B., Pihlaja K. & Pulkkinen P. 2006: Seeing the trees for the leaves: oaks as mosaics for a host-specific moth. *Oikos* **113**: 106–120.

Sarfraz R.N., Dosdall L.M. & Keddie A.B. 2009: Bottom-up effects of host plant nutritional quality on Plutella xylostella (Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. *Eur. J. Entomol.* **106**: 583–594.

Stein S.J. & Price P.W. 1995: Relative effects of plant resistance and natural enemies by plant development age on sawfly (Hymenoptera: Tenthredinidae) preference and performance. *Environ. Entomol.* **24**: 909–916.

Suomela J. & Ayres M.P. 1994: Within tree and among-tree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos* **70**: 212–222.

Thompson J.N. & Pellmyr O. 1991: Evolution of oviposition behaviour and host preference in Lepidoptera. *Annu. Rev. Entomol.* **36**: 65–89.

Trager M.D., Boyd B.M., Daniels J.C. & Pence J.A. 2009: Host plant selection, larval survival and reproductive phenology in Megathymus yuccae (Lepidoptera: Hesperiidae). *Environ. Entomol.* **38**: 1211–1218.

Vuorisalo T., Walls M., Niemela P. & Kuitunene H. 1989: Factors affecting mosaic distribution of galls of an eriophyid mite, Eriophyes laevis, in alder, Alnus glutinosa. *Oikos* **55**: 370–374.

Wallin K.F. & Raffa K.F. 1998: Association of within-tree jack pine budworm feeding patterns with canopy level and within needle variation of water, nutrient and monoterpene concentration. *Can. J. Forest Res.* **28**: 228–233.

Zehnder C.B., Stodola K.W., Joyce B.L., Egetter D., Cooper R.J. & Hunter M.D. 2009: Elevational and seasonal variation in the foliar quality and arthropod community of Acer pensylvanicum. *Environ. Entomol.* **38**: 1161–1167.

Received February 26, 2010; revised and accepted June 25, 2010

Appendix

Clutch size may vary, more or less, depending on circumstances, whatever the latter happen to be. Yet, for simplicity the distribution of clutch sizes within a given sample of host units may be restricted to its average value. In turn, the latter may even be approximated by the nearest integer. According to this simplification (the original form of the "Melba" procedure, see Béguinot, 2009a, b), any acceptable host unit that receives v visits by an ovipositing female will host $(v.n_c)$ eggs.

However, when clutch size is low, more precision may be desirable and then, the decimals of the average clutch-size have to be considered also (Béguinot, 2008). For example, let us consider the case where a mother may lay either 1 or 2 eggs in a single bout during a visit to an acceptable host unit. Then, let x ($x \leq 1$) and $(1–x)$ be the respective proportions of mothers laying either 2 or 1 eggs per visit, which results in an average clutch size of $(1+x)$. Now, for an acceptable host unit that receives v visits from gravid mothers, the respective proportions of deposits of either 1 or 2 eggs can be predicted by means of a binomial distribution. The probability of an acceptable host unit receiving k deposits of 2 eggs and $(v–k)$ deposits of 1 egg (that is a total of $(v+k)$ eggs) is thus:

$$p(v,x,k) = C(v,k)\ x^k\ (1–x)^{(v–k)} \text{ with } C(v,k) = v!/k!/(v–k)!$$

This binomial distribution, parameterised in x, should therefore substitute for the single value $(v.n_c)$ when evaluating the total number of eggs supported by acceptable host units that receive v visits. Accordingly, the theoretical distribution, $\Pi(\eta)$, of the total number of eggs per host unit, which was parameterised in n_c and α in the original procedure (Béguinot, 2009a, b), is now parameterised in x and α, with the resulting average clutch size, n_c, being equal to $(1+x)$. Then, the best fit between the recorded and corresponding theoretical distributions, $\Pi(\eta)$, will be now in terms of adaptable parameters x and α, instead of n_c and α. In addition, as mentioned above, artefacts (mines or galls) resulting from successfully hatched eggs may be recorded and considered, instead of eggs themselves, with the only restriction that the term “clutch size” be applied to the artefacts rather than to the original clutch of eggs.