

WOULD CLUTCH SIZE DECREASE WITH HIGHER LOCAL-DENSITY OF MOTHERS AND/OR LOWER QUALITY OF THE HOST IN LEAF-MINING MOTHS? A CASE STUDY WITH THREE COMMON LEPIDOPTERAN LEAF-MINERS

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RÉSUMÉ. — *La taille de la ponte décroît-elle avec une plus forte densité locale des mères et/ou une plus faible qualité de l'hôte chez les lépidoptères mineurs de feuilles ? Le cas de trois espèces communes.* — Chez les insectes herbivores à développement larvaire sessile (tels que les insectes mineurs ou cécidogènes de la feuille-hôte), le contrôle de la taille de ponte élémentaire permet aux mères de viser à un approvisionnement optimal pour les futures larves. La taille de ponte élémentaire peut ainsi constituer une réponse adaptative aussi bien (i) au niveau moyen de qualité des feuilles de l'hôte, destinées à la consommation des larves, que (ii) à la densité locale des mères, de sorte à éviter ou au moins limiter la compétition entre larves exploitant une même feuille-hôte. Cependant, un tel contrôle de la taille de ponte suppose évidemment que les mères pondueuses soient en capacité préalable de reconnaître le niveau moyen de qualité foliaire de l'individu hôte et/ou d'apprécier approximativement la densité des mères dans leur voisinage et d'être en outre motivées à tenir compte de ces paramètres. Cette hypothétique aptitude des mères à adapter la taille de ponte en fonction des facteurs précités mérite donc d'être testée au sein de la guildes des insectes fonceurs de mines ou inducteurs de galles foliaires. Considérant ici trois espèces communes de micro-lépidoptères formant des mines foliaires, on montre que la taille de ponte élémentaire demeure substantiellement indépendante aussi bien de la qualité moyenne des feuilles-hôtes (appréciée au moyen de l'estimation de la proportion ' α ' de feuilles potentiellement acceptables par les mères) que de la densité des mères (appréciée indirectement au moyen de la densité ' μ ' de visites probatoires reçues par feuille). Pour ces trois espèces au moins, les précautions maternelles apparaissent donc se limiter au choix de l'espèce-hôte puis à la sélection des feuilles considérées acceptables pour ponte, au sein de l'individu-hôte, sans que la taille de ponte soit elle-même un levier adaptatif.

SUMMARY. — In herbivorous insects having a concealed larval stage, such as leaf-miners and leaf-gallers, regulating the size of the clutch may be a way for ovipositing mothers to ensure optimal resource availability for future offspring. Clutch size regulation may thus serve as an adaptive response to either (i) the average level of quality of host leaves as a food resource for offspring, or (ii) to the local density of conspecific mothers, so as to prevent scramble competition among larvae. Accordingly, clutch size regulation would imply the capacity for mothers (i) to recognize the level of leaf quality through an appropriate probing process and/or (ii) to evaluate the local density of conspecific mothers. Clutch size regulation that may possibly result from either of the two preceding factors thus needs to be addressed within the guilds of mining and galling insects. In three common leaf-mining moth species I observed that the average clutch size ' n_c ' is only weakly, non significantly related to (i) the leaf-acceptance ratio ' α ' of mothers, which mirrors the average foliar quality of the host-individual, and (ii) the density ' μ ' of mothers making visits to host leaves, which mirrors the local density of conspecific mothers. Therefore, clutch size is not related to the local density of conspecific mothers or the average foliar quality of host individuals for these three mining species.

Maternal care, especially *pre-hatching* maternal care is widespread among herbivorous insects because, usually, the larval stage is hardly mobile and the diet of larvae is often limited

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to a rather narrow range of host plant species (Jolivet, 1983; Dajoz, 1993). Accordingly, ovipositing mothers generally choose (i) the best resource for offspring by *selecting the appropriate support on which the eggs are laid*, and (ii) they reduce the risk of scramble competition within the brood by *limiting clutch size*. This is especially the case for insects with a larval stage concealed within a narrow part of the host plant, such as *mine-forming* or *gall-inducing* insects (Faeth, 1991).

For mothers of leaf-mining/galling species, selection of the substrate on which eggs are laid consists not only of reaching the proper host species, but also of selecting the most appropriate host individual and, eventually, within the individual, the more promising sub-sets of host leaves that will satisfy the minimum requirements of mothers after careful probing. The latter choice is often decisive since leaf quality may vary at least as much within as among individual hosts (Schultz *et al.*, 1982; Schultz, 1983; Boomsma *et al.*, 1987; Kozlov & Koricheva, 1991; Roslin *et al.*, 2006; Gripenberg, 2007). Subsequently, as soon as the probed leaf is accepted, mothers may make a decision about clutch size, i.e. the number of eggs laid after a visit to a positively probed host leaf.

In quantitative terms, let

- ‘ α ’ be the proportion of host leaves that satisfy the requirements of egg-laying mothers’ and thus would be accepted if visited and probed, i.e. the “*leaf-acceptance ratio*”;

- ‘ n_c ’ be “*mines-clutch size*”, i.e. the average number of singly occupied mines resulting from the hatching of a clutch of eggs, the notion of “clutch” being then related to the outcome of a definite oviposition event (Fordyce, 2005).

This “pre-hatching maternal care”, involving limitations on both the leaf acceptance ratio ‘ α ’ and the clutch size ‘ n_c ’, is maternal investment because both limitations contribute to slow down the progress of oviposition, which may thus become a constraint on ovipositional efficiency in a context of limited time expenditure (Reavey & Gaston, 1991).

Three factors may possibly constrain clutch size, so as to prevent excessive scramble competition among future larvae occupying a single host leaf. Mothers may be induced to *reduce their clutch size* in response to:

- (i) an increasing density of eggs already previously deposited on leaves,
- (ii) an increasing local density of conspecific mothers, and
- (iii) a decreasing average quality of the leaves of the host individual under consideration (i.e. decreasing α value).

The *first* factor would imply the ability of mothers to recognize conspecific eggs, a capacity that has been acknowledged in many groups of insects, but is yet considered unlikely among most tiny leaf-miner species (Sugimoto, 1980; Quiring & McNeil, 1987; Auerbach & Simberloff, 1989; Faeth, 1990; Green & Prokopy, 1991; Craig *et al.*, 2000; Cronin *et al.*, 2001; Kagata & Ohgushi, 2002; Cornelissen & Stiling, 2008; Digweed, 2006; Gripenberg, 2007; Gripenberg & Roslin, 2005; Tack *et al.*, 2009; Tack, 2010; but see Kagata & Ogushi, 2001)².

The *second* factor implies that the co-occurrence of conspecific females in their immediate vicinity may be recorded by mothers thereby inducing them to reduce their clutch size (Quiring & McNeil, 1984; Bultman & Faeth, 1986). Mothers may be able to recognize the presence of conspecific females in their neighbourhood more easily than minute eggs on leaves. Accordingly, the possibility exists that mothers reduce their clutch size due to increasing density of neighbouring conspecifics. However, this hypothesis has less frequently been addressed, since local density of mothers is difficult to assess in the field.

The *third* hypothesis, i.e. the capacity of leaf-mining/galling mothers to test host-leaves quality, was already addressed and positively documented, as mentioned above (see also Strong *et al.* 1984; Cornelissen & Stiling 2008; Tack 2010; Béguinot 2009a,b, 2011, 2012a,b).

² Would mothers actually be able to recognize previously deposited eggs and thereby avoid multiple egg clutches successively deposited on a single leaf, then the variance to the mean ratio for the distribution of the numbers of eggs per leaf, *within the set of potentially acceptable* leaves, would be close to zero or, at least, much lower than 1. This has been checked beforehand and proved never to be the case here.

Now, probing and selecting host-leaves according to their quality *before* oviposition is one thing and the hypothetical adaptation of the clutch size to the level of leaf quality is another, which remains to be tested.

The fine-tuning of maternal behaviour, involving the adaptation of clutch size to the local density of conspecifics and/or average leaf quality of the host may have obvious implications for density-dependent regulation of insect populations. Although the densities of leaf-miners under field conditions seems usually low with limited risk of scramble competition (Cornelissen & Stiling, 2008; Tack *et al.*, 2009; Tack, 2010), the co-occurrence of several larvae exploiting a single host-leaf has, yet, also been reported and considered detrimental for the development and survival of progeny in pest outbreaks (Auerbach *et al.*, 1995; Tack *et al.*, 2009).

The objective of this study was to address hypothesis (ii) and (iii): the possibility that egg-laying females would significantly decrease their average clutch size ' n_c ' in response to an increasing local density of conspecific females, and/or in response to the decreasing average leaf quality of the host. For this purpose, we considered three moth-species (*Phyllonorycter maestingella*, *Phyllonorycter esperella*, *Tischeria ekebladella*) mining three major European host tree species (beech, hornbeam and oak, respectively). For each moth species, several sites (from 6 to 22) were selected in consideration of their relatively high (while still non-eruptive) density of mines per leaf, the density varying in a range sufficiently large to test efficiently for the possible influence of the density of neighbouring conspecific females.

METHODS

ESTIMATION OF THE QUANTITATIVE TRAITS OF EGG-LAYING BEHAVIOUR

The evaluations of behaviour parameters, α and n_c , face important practical difficulties, since none of these parameters may be easily observable directly in the field, especially considering tiny insects as leaf-miners/gallers. Moreover, when mining/galling impact levels are recorded, time for monitoring the corresponding driving parameters α , n_c is already long gone.

Accordingly, a specific method, the "Melba procedure" was derived so as to overcome these practical difficulties. This procedure conveniently provides *indirect* estimations of α , n_c on the only basis of the easily recorded distribution of the number of mines/galls per leaf, $\Pi(\eta)$, within a sampled portion of the host canopy. In short (see the Appendix for basic information on the rationale for the procedure (and also Béguinot 2009a,b, 2011, 2012a,b for further details), this procedure relates the hard-to-observe parameters α and n_c to the field-recorded distribution $\Pi(\eta)$ of the numbers of leaves according to the number η of either eggs or mines they support.

Briefly, the procedure is based on the fact that, thanks to acceptable simplifying assumptions (Béguinot, 2012a, b), the distribution $\Pi(\eta)$ should theoretically provide a specific equation with α and n_c as the adaptable parameters. Conversely, the inferred values for α and n_c are those that yield the best fit between the recorded distribution $\Pi(\eta)$ and the corresponding theoretical distributions $\Pi(\eta)$, parameterized in terms of α and n_c . In turn, the estimated average number ' μ ' of mothers' probing visits received per leaf is derived by dividing the recorded average number of mines per leaf by ($\alpha.n_c$). The 'Melba' procedure is rapid and easy to run and, therefore, reveals particularly appropriate when relatively large series of samples are to be analysed, as is the case here.

FIELD DATA

The possibility of mothers decreasing their clutch size as a response to an increase in local density is more likely when the local density of females - and the resulting value of the density μ of probing visits - is high. Accordingly, relatively high levels of mining-impact were selected for this study (from 0.10 to 0.88 mines per leaf), so that the average number μ of maternal probing visits to host leaves is 0.1 - 1.5 probing visit(s) per leaf. This is just below the threshold level for the occurrence of nascent eruptive outbreaks, (i.e. impacts > 1 mine/leaf: see Auerbach *et al.* (1995)). Note that such outbreaks seem quite infrequent in the three species considered here (personal observations). Even slightly lower ranges of impacts, say between 0.1 and 1.0 mine per leaf, as is the case here, are rare.

The distribution $\Pi(\eta)$ of mines among host-leaves was carefully assessed within the lower part of the host canopy (typically between one and two m height) for 34 trees, each from a different locality, mainly in south Burgundy (see Appendix for details). Trees were haphazardly selected in each locality, among those supporting at least 0.1 mine per leaf within the sampled branch(es). A total of 14,118 leaves were examined, supporting 4502 mines. The distribution of samples and observations according to leaf-mining species was as follows: *Phyllonorycter maestingella* (Müller, 1764) on *Fagus sylvatica* L.: 22 stands, a total of 10,146 leaves with 2726 mines; *Phyllonorycter esperella* (Goeze, 1783) on *Carpinus betulus* L.: 6 stands, 2473 leaves with 1355 mines; *Tischeria ekebladella* (Bjerkander, 1795) on *Quercus sessiliflora* (Sm.): 6 stands, 1499 leaves with 421 mines.

RESULTS

DEPENDENCE OF CLUTCH SIZE UPON THE DENSITY OF MOTHERS' PROBING VISITS

The average clutch size n_c (understand hereafter as the average “mines clutch-size”, as defined above) is plotted against the average number μ of mothers' probing visits per leaf, for each of the three studied leaf-mining moths, *Phyllonorycter esperella*, *Phyllonorycter maestingella* and *Tischeria ekebladella* (Fig. 1).

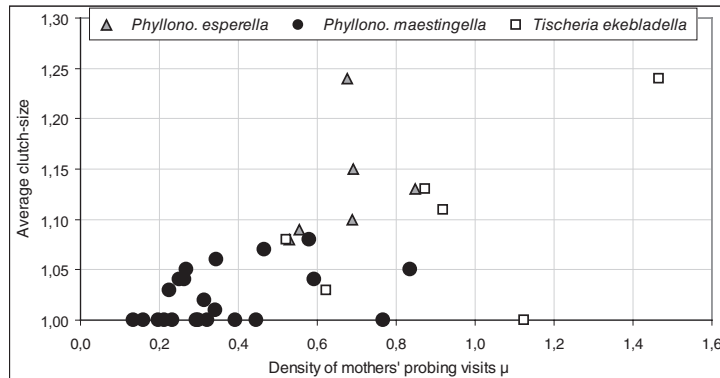


Figure 1.—The average clutch size n_c plotted against the average number of mothers' probing visits per leaf μ , for three species of leaf-mining moths *Phyllonorycter esperella*, *Phyllonorycter maestingella* and *Tischeria ekebladella*.

The tested hypothesis is that the average clutch-size n_c would *decrease* with increasing values of the density μ of mothers' probing visits to host-leaves, i.e. n_c *decreasing* with growing local density of mothers. This hypothesis is clearly not supported. On the contrary, n_c tends to slightly increase with growing μ for each of the three species, although this trend is not statistically significant in any case. For *Phyllonorycter esperella*, $r^2 = 0.13$, d.f. = 4, $p > 0.10$; for *Phyllonorycter maestingella*, $r^2 = 0.14$, d.f. = 22, $p > 0.10$ and for *Tischeria ekebladella* $r^2 = 0.33$, d.f. = 4, $p > 0.10$.

DEPENDENCE OF CLUTCH SIZE UPON THE AVERAGE FOLIAR QUALITY OF HOST INDIVIDUALS

The average clutch size n_c is plotted against the leaf acceptance ratio α , for each of the three studied leaf-mining moths, *Phyllonorycter esperella*, *Phyllonorycter maestingella* and *Tischeria ekebladella* (Fig. 2).

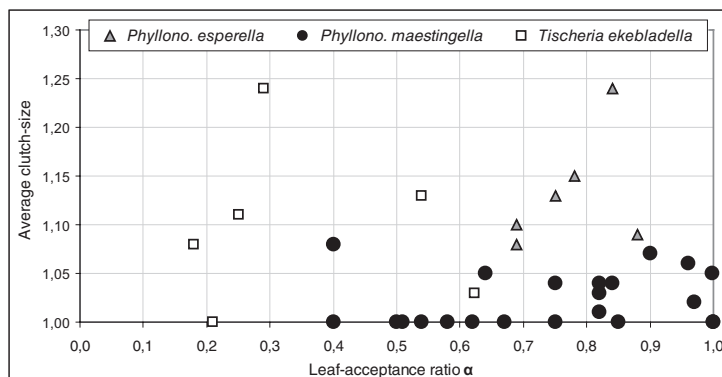


Figure 2.—The average clutch size n_c plotted against the leaf-acceptance ratio α for three species of leaf-mining moths *Phyllonorycter esperella*, *Phyllonorycter maestingella* and *Tischeria ekebladella*

The tested hypothesis is that the average clutch-size n_c would *increase* with increasing values of the leaf acceptance ratio α , i.e. n_c *increasing* with improved leaf quality according to mothers' requirements. This hypothesis is clearly not supported. On the contrary, n_c tends to slightly (although non significantly) decrease with increasing α for *Phyllonorycter maestingella* and *Tischeria ekebladella*. For *Phyllonorycter maestingella*: $r^2 = 0.03$, d.f. = 22, $p > 0.10$ and for *Tischeria ekebladella*: $r^2 = 0.005$, d.f. = 4, $p > 0.10$). For *Phyllonorycter esperella*, a positive correlation is disclosed but remains, yet, non significant: $r^2 = 0.18$, d.f. = 4, $p > 0.10$.

DISCUSSION

Clutch size varies considerably among herbivorous insects, but is generally smaller for internal than for external feeders (Connor & Taverner, 1997). In leaf miners, the average clutch size usually ranges from one to a few (Quiring & McNeil, 1987; Reavey & Gaston, 1991; Connor *et al.*, 1994; Auerbach *et al.*, 1995; Cappuccino, 2000; Kagata & Ogushi, 2001, 2002; Sugiura & Yamazaki, 2003). Most often clutch size is either one or two, with an average value often less than 1.5 in leaf-mining flies and moths (Béguinot, 2011, 2012a). The possible range of variation in clutch size in leaf-mining insects is thus reduced, but *a priori* it might still be negatively influenced by local density of conspecific mothers or by decreasing average foliar quality of the host individual. Yet, the results presented in Figures 1 and 2 do not support these hypotheses in the three moth species studied here. This is not really surprising. The lack of capacity of mothers to recognize or react to this factor (by relaxing their selectivity) has already been reported (Béguinot, 2011, 2012b). As concerns average quality of the foliage of the host individual there is no doubt that mothers are able to probe leaf quality. But because mothers only select those host leaves that satisfy their own requirements of quality for subsequent ovipositions, they already account for the average level of quality of the foliage (through the proportion α of leaves recognized as being potentially acceptable by mothers).

Clutch size n_c is largely independent of both (i) the density μ of mother probing visits to leaves and (ii) the leaf-acceptance ratio α for the three mining moths species. As α was also shown to be independent of μ (Béguinot, 2012b), the three parameters α , n_c , μ , are mutually independent. Now, the level 'I' of impact of leaf-mining on the host (in terms of the average number of mines per leaf) is the product of these three parameters ($I = \alpha \cdot n_c \cdot \mu$).

The mutual independence of α , n_c , μ , entails that many different combinations of these parameters would similarly result in a given level of mining impact. Therefore, understanding the significance of a given level of impact of miners on a tree, in terms of the causes involved, will imperatively require the independent estimates of each of the three parameters α , n_c , μ .

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APPENDIX A BRIEF SUMMARY OF THE 'MELBA' PROCEDURE

THE THREE BASIC ASSUMPTIONS UNDERLYING THE 'MELBA' PROCEDURE

The following points are assumed:

(i) a random distribution of mothers' probing visits among host-leaves (of course, this will no longer be the case for efficient visits, i.e. concluded by ovipositions, as soon as not all leaves are acceptable, that is $\alpha < 1$). This first assumption is commonly referred to (Sugimoto, 1980: 15; Connor *et al.*, 1997; Kuhlmann *et al.*, 1998; Wool & Ben-Zvi, 1998; Kagata & Ohgushi, 2002; Vos & Hemerik, 2003; Ives & Godfray, 2006).

(ii) *Equi-probability* of mothers' probing visits among host leaves; the differences which may actually exist between the probabilities of visits, for example in relation to variation in the individual size of leaves, are neglected as a first approximation, as far as all these leaves belong to the *same type* and represent the same 'space module' for the insect, according to the observations of Digweed (2006), the argumentation of Kuczynski & Skoracka (2005) and Gripenberg & Roslin (2005). Indeed, such simplifications are commonly used in models, see for example Kagata & Ohgushi (2004) for leaf-miners or Kuczynski & Skoracka (2005) for leaf-gallers.

(iii) *Insect species are only* considered, when mothers *do not* decidedly avoid nor restrict oviposition to leaves already visited and used for egg-laying previously by themselves or conspecifics (Sugimoto, 1980: 14; Quring & McNeil, 1987; Auerbach & Simberloff, 1989; Faeth, 1990; Green & Prokopy, 1991; Craig *et al.*, 2000; Cronin *et al.* 2001; Kagata & Ohgushi, 2002; Cornelissen & Stiling, 2008; Digweed, 2006; Gripenberg, 2007; Gripenberg & Roslin 2005; Tack *et al.*, 2009; Tack, 2010). This last condition, *not* having a general applicability, has therefore to be systematically checked first. In particular those cases where the distribution of the recorded numbers of eggs per acceptable unit would show a variance sub-null or, at least, much lower than the mean should be excluded from the Melba procedure, especially when female density and oviposition pressure are high, since that would suggest avoidance of cumulative egg-laying' bouts on a same unit.

A BRIEF SUMMARY OF THE RATIONAL FOR THE MELBA PROCEDURE

According to the two first assumptions above (i.e. random distribution and equi-probability of probing visits among leaves), the distribution of the number of *probing* visits received per host-leaf would ideally conform to a Poisson law. Among all probing visits, only those received by *potentially acceptable* leaves, according to mothers' requirements, are concluded with an egg-clutch deposit: the reason why, at the difference of the distribution of visits, the distribution of eggs deposits (and associated offspring) within the whole set of sampled leaves does *not* conform to, and more aggregated than Poisson.

Conformity to the Poisson law of the distribution of egg-deposits is expected *only* within the set of *potentially acceptable* leaves (*on proviso* of the third assumption above being satisfied, since then, mothers do *not avoid* ovipositing on leaves already previously use for egg-laying).

Then, let $\Pi(v)$ be the proportion of leaves that received v "efficient" visits (each one concluded by an egg-clutch deposit), among the *whole set* of n_u sampled leaves.

For $v \neq 0$, i.e. leaves having received at least one "efficient" visit, only the acceptable ones are concerned, by definition. Then, $\Pi(v)$ is given by applying the Poisson law to the set of *potentially acceptable* host-leaves *only*, which represent a proportion α of the whole set of leaves. Accordingly, for $v \neq 0$:

$$\Pi(v) = \alpha \cdot \exp(-\mu) \cdot \mu^v / v! \quad [1] \quad (v \neq 0)$$

For $v = 0$, i.e. leaves having received no efficient visit, two contributions are to be considered: all the unacceptable leaves (proportion $(1 - \alpha)$) and also those acceptable leaves that, by chance, have received no visits:

$$\Pi(v) = 1 - \alpha + \alpha \cdot \exp(-\mu) \quad [1'] \quad (for \ v = 0)$$

with μ as the average number of probing visits received per host-leaf (μ is, as well, the average number of "efficient" visits received per potentially acceptable leaf).

Now, what may be observed and quoted *a posteriori* in the field is not the number of efficient visits per leaf but their direct material consequences in term of the number η per host-leaves with either deposited eggs (if possibly recorded at the time of field record) or resulting mines.

If *deposited eggs* may directly be recorded in the field, then n_c corresponds to the usual definition of clutch size: the number of eggs deposited in a single bout.

If only the (mono-occupied) *mines* may be recorded in practice, as is the case here, then n_c will represent the average number of mines resulting from one clutch of eggs (here referred to as the 'mines' clutch-size). In this case, no inference, of course, will be made from the estimated mines clutch size towards the genuine eggs clutch size itself, in the absence of further information on the hatching ratio of eggs.

The number η of eggs per leaf is thus $\eta = v \cdot n_c$, with v as the number of visits received and n_c being either the 'eggs' or (here) the 'mines' clutch-size. The proportion $\Pi(\eta)$ of host-leaves supporting η eggs or mines, among the whole sample of n_u host-leaves, is therefore obtained by substituting η/n_c to v , in equations [1] & [1']:

*for any positive values of (η/n_c) , i.e. for acceptable leaves visited at least once:

$$\Pi(\eta) = \alpha \cdot \exp(-\mu) \cdot \mu^{(\eta/n_c)} / (\eta/n_c)! \quad [2]$$

* and for $\eta = 0$:

$$\Pi(0) = 1 - \alpha + \alpha \cdot \exp(-\mu) \quad [2']$$

The average number μ of "efficient" visits per acceptable leaf, is given by:

$$\mu = (n_c/n_c) / (\alpha \cdot n_u) = n_c / (\alpha \cdot n_c \cdot n_u) \quad [3]$$

with n_c as the total number of eggs (or, here, the total number of mines) within the whole studied sample of n_u potential host-leaves (acceptable or not); note that considering either eggs or associated artefacts does not modify neither (n_c/n_c) nor μ , since n_c is the clutch-size applied to either eggs or mines. Therefore, the acceptance ratio α also remains unchanged since $\alpha = (n_c/n_c) / (\mu \cdot n_u)$.

The estimation of both n_c and α is obtained by comparing the field-recorded distribution $\Pi(\eta)$ (established for either eggs or their resulting artefacts) to the corresponding computed distribution $\Pi(\eta)$.

In practice, this estimation is obtained iteratively, seeking for the set of values $\{n_c \ \& \ \alpha\}$ which leads to the best fit between *field-recorded* and *computed* distributions $\Pi(\eta)$. It should only be well defined when n_c corresponds to either the *eggs'* or to the *mines'* clutch-size, depending upon what field recorded data is implemented in the Melba procedure.

In turn, the average number μ of mothers probing visits per host-leaf is given by equation [3]: $\mu = (n_c/n_u) / (\alpha \cdot n_c)$. Note that this relation provides no assumption regarding the existence of a correlation between μ on the one hand and α or n_c on the other hand. Indeed, this expression only defines the mining incidence, n_c/n_u , as the consequence of the separate and multiplicative contributions of α , n_c , μ : $n_c/n_u = \alpha \cdot n_c \cdot \mu$. It is the aim of this paper to test for the existence of a correlation between μ and either α or n_c .

A complete description of the Melba procedure, including the statistics of the Melba estimates, is provided in Béguinot (2012 a).

Localities and dates of samplings

| <i>Fagus sylvatica</i> | |
|-----------------------------|------------|
| 19-Chamberet | 29 10 2005 |
| 19-Chamberet | 30 10 2005 |
| 19-Chamberet | 12 08 2009 |
| 52 - Auberive | 29 09 2008 |
| 71 Saint Léger sous Beuvray | 3 09 2005 |
| 71 Saint Pierre de Varennes | 11 11 2005 |
| 71-Antully | 11 10 2005 |
| 71-Antully | 26 10 2005 |
| 71-Antully | 26 10 2005 |
| 71-Antully | 26 10 2005 |
| 71-Les Bizots | 23 09 2008 |
| 71-Montchanin | 23 08 2005 |
| 71-Montchanin | 23 08 2005 |
| 71-Montchanin | 5 09 2005 |
| 71-Montchanin | 27 09 2005 |
| 71-Montchanin | 19 10 2005 |
| 71-Perreuil | 2 10 2005 |
| 71-Perreuil | 2 10 2005 |
| 71-Saint Sernin du Bois | 14 10 2005 |
| 71-Saint Sernin du Bois | 14 10 2005 |
| 71-Saint Sernin du Bois | 14 10 2005 |
| 71 Saint Léger sous Beuvray | 3 09 2005 |

| <i>Carpinus betulus</i> | |
|-------------------------|------------|
| 71-Blanzy | 12 10 2008 |
| 71-Saint Sernin du Bois | 11 09 2008 |
| 71-Saint Sernin du Bois | 10 07 2009 |
| 71-Saint Sernin du Bois | 10 07 2009 |
| 71-Saint Sernin du Bois | 10 07 2009 |
| 71-Saint Sernin du Bois | 10 07 2009 |
| 71-Saint Sernin du Bois | 10 07 2009 |

| <i>Quercus sessiliflora</i> | |
|-----------------------------|------------|
| 19-Chamberet | 30 10 2005 |
| 71-Fley | 6 09 2009 |
| 71-Fley | 6 09 2009 |
| 71-Saint Sernin du Bois | 14 10 2005 |
| 71 Saint Léger sous Beuvray | 28 08 2005 |
| 71 Saint Léger sous Beuvray | 28 08 2005 |