

Putting more eggs in the best basket: clutch-size regulation in the comma butterfly

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Abstract. 1. Many studies have identified different factors influencing clutch-size regulation, primarily within various groups of insects. One prediction is that ovipositing females should increase clutch size with host quality. However, in many studies it is not clear whether ovipositing females are responding to host quality or quantity.

2. Females of the polyphagous comma butterfly, *Polygonia c-album* (L.), were allowed to oviposit on two hosts differing greatly in quality: the preferred host, stinging nettle (*Urtica dioica* L.), and the low-ranked host, birch (*Betula pubescens* Ehrh). Ovipositing females were observed visually and clutch sizes were recorded. The experiment was repeated in three different years; in total, 938 observations of oviposition events were made.

3. In all three years, females ovipositing on *U. dioica* laid larger clutches (median 1.6–1.85) compared with females ovipositing on *B. pubescens* (median 1.0–1.3). The difference was significant in two out of three years and when all three years were pooled.

4. Thus, *P. c-album* females exhibit clutch-size regulation, with larger clutches on better hosts. It is suggested that the proximate mechanism is likely to be a response to the same stimuli used for female ranking of host plants in the preference hierarchy.

Key words. Batches, host quality, life history, performance, preference, reproductive output.

Introduction

The reproductive schedule over time is a central life-history trait in animals, with consequences for shaping other life-history traits and, because of the close connections between life history and fitness, also for shaping other adaptive traits. Clutch size is very important in shaping this schedule of reproduction 'because it represents a proportion of a female's potential fecundity that is invested in a single oviposition event' (Fordyce & Nice, 2004). A large body of research has dealt with different factors influencing clutch-size regulation within various groups of insects. Among these factors are resource size (Godfray, 1986; Zaviezo & Mills, 2000), resource density (Tsubaki, 1995),

female age (Freese & Zwölfer, 1996; D'az-Fleischer & Aluja, 2003), female egg load (Tatar, 1991; Visser, 1996; Monks & Kelly, 2003), time since last oviposition (Agnew & Singer, 2000; Janz & Thompson, 2002), previous female experience (Monks & Kelly, 2003), competition between females and larvae (Vasconcellos-Neto & Monteiro, 1993; Tsubaki, 1995; Visser, 1996), and benefits from aggregated larval feeding (Fordyce & Nice, 2004).

One of the factors that should contribute most strongly to clutch-size variation and regulation is resource size. A larger host can support more larvae and hence should allow the female to lay larger clutches. This type of clutch-size regulation has been demonstrated repeatedly in insects, primarily in parasitoids, but also among butterflies (Damman, 1991; Vasconcellos-Neto & Monteiro, 1993; Zaviezo & Mills, 2000).

Another more subtle type of regulation is response to host quality *per se*, defined here as laying larger clutches of eggs on hosts with similar size (providing similar amounts

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of resources) but that support better performance for the individual larvae in terms of survival, growth, reproduction as adults, and ultimately fitness. This is a strategy that should increase fitness for ovipositing females in insects that do not lay large batches and are time limited. It will decrease total search time and increase reproductive output while still assuring high offspring performance by placing more eggs on high-quality hosts. The ability of female insect herbivores to adjust their reproductive output in response to host-plant quality is not well understood (Leather, 2002).

Clutch size models, predicting that ovipositing females should increase clutch size with host quality (Skinner, 1985; Godfray, 1987; Wilson & Lessells, 1994), as well as empirical studies (Damman, 1991), have in fact often used the terms quality and quantity interchangeably. Damman (1991) 'looked at clutch size as a function of plant quality ...' and found that 'Females laid significantly larger clutches on pawpaws with many leaves than they did on pawpaws with few leaves'. In studies claiming to have demonstrated a difference in clutch size in response to host quality it is unclear whether the differences under investigation have in fact been resource quantity or quality (i.e. Pilsen & Rausher, 1988; Freese & Zwölfer, 1996). Pilsen and Rausher (1988) performed a field experiment to determine whether females of the pipewine swallowtail butterfly '... lay larger clutches on plants of higher quality.' and from the results suggest that '... females tend to place larger clusters on plants with more edible foliage ...'. Freese and Zwölfer (1996) performed oviposition experiments on thistle gall flies to show that the quality of the oviposition site had an influence on clutch size and concluded that the diameter (size) of the internal bud had a significant effect on clutch size. The reason for this confusion is probably that for many species oviposition site 'quality' – in the wider sense of offspring performance – is in fact often determined by host size, such as in parasitoids and other food-limited species. However, this is not always the case. For plant-feeding insects, host quality often varies independently of host size, because plants are chemically variable between species, individuals, and tissue types (e.g. Janz *et al.*, 1994; Singer & Lee, 2000; D'az-Fleischer & Aluja, 2003).

One study where clutch size was unequivocally tested in response to host quality was performed on the tephritid fly *Anastrepha ludens* by Díaz-Fleischer and Aluja, 2003). They found a negative response to host quality, where females laid larger clutches on low quality hosts.

The present study was conducted to test if females of the polyphagous comma butterfly, *Polygonia c-album* (L.), exhibit clutch-size regulation in response to host-plant quality by laying larger clutches on better hosts (subsequently referred to as 'positive clutch-size regulation'). The comma butterfly lays from one up to a few eggs at each oviposition event and has the capacity to lay several hundred eggs in total. This oviposition pattern is likely to put females under time constraints, especially if the typically unpredictable weather conditions during the early

spring flight period in Sweden are considered (Wiklund & Persson, 1983). In the test reported here, stinging nettle, *Urtica dioica* L., and birch, *Betula pubescens* Ehrh., were utilised, because it is well known that these host plants differ in quality. Stinging nettle is a superior host in terms of offspring performance and is preferred by females (Nylin & Janz, 1993; Janz *et al.*, 1994). Both hosts are used regularly in the field, together with several other hosts that are available in the study region.

Materials and methods

Study species

The study was carried out using Swedish females of the comma butterfly, *Polygonia c-album*. This species is distributed all across the Palearctic region, and populations differ in host-plant specificity, with the Swedish population being highly polyphagous (Janz & Nylin, 1997). It is capable of using plants from seven families in four orders as hosts (Urticaceae, Ulmaceae, and Cannabidaceae in Urticales, Salicaceae in Salicales, Betulaceae, and Corylaceae in Fagales, and Grossulariaceae in Rosales). In this experiment, two different host plants, stinging nettle, *Urtica dioica*, and birch, *Betula pubescens*, were used. Rearing experiments have shown that *U. dioica* is a superior host to *B. pubescens*, with 30–50% faster larval growth, shorter development time, heavier pupae and higher survival (Janz *et al.*, 1994).

The females of *P. c-album* typically lay their eggs singly or in small clutches on the leaves of the host plants. The larvae feed solitarily on the leaves and eventually leave the host to pupate. The butterfly hibernates in the adult stage but can, under favorable circumstances, produce a second, directly developing generation in the summer.

Experimental procedures

The butterflies used in the experiments were bred from 18 females caught in the wild near Stockholm, Sweden. Females of the summer form, without adult hibernation diapause, were produced following the procedure described by Nylin (1989, 1992); larvae were reared on cuttings of *U. dioica* or *Salix caprea*, willow, in a regime of increasing day length and temperature. The host plant used for rearing does not affect the preferences of the resulting adult females (Nylin & Janz, 1996). All butterflies were marked individually and subsequently kept in large flight cages for mating. Mated females were put in individual flight cages (0.5 m × 0.5 m × 0.5 m), where the oviposition experiment was conducted. Each flight cage was lit from above by a 75-W light bulb between 8.30 and 16.30 hours. The cage floor was covered with moist paper, and the butterflies were fed with a mixture of sugar and water from a sponge placed in the cage directly under the light. The host plants

used in the experiment was presented as stalks standing in bottles of water.

Host plants were circulated among all the different cages (to prevent cage effects) throughout the experiment and were replaced at first sign of wilting. The relative position of the plants inside the cages was changed frequently during the observation sessions to prevent position effects (several times daily in cages with much oviposition activity and in all cages at least daily).

Individual females were assigned randomly to perform a no-choice test with equal-sized cuttings of either *U. dioica* or *B. pubescens*; tests with either host plant were run simultaneously in parallel cages. This was carried out to ensure that the females were of equal age and state of motivation. Ovipositing females were observed visually and clutch sizes were recorded over a period of 3–6 days, depending on oviposition rate. Because several flight cages were in use at the same time it was not possible to observe all cages continuously. An observation scheme was therefore adopted, where the observer walked a set path along the cages. A standardised observation period was started whenever an oviposition event was observed. The female was then observed for 3 min and all oviposition events during that time period were noted. Eggs were taken to be of the same clutch if the female did not take off between laying the eggs but moved around on the plant. In total, 938 oviposition events were observed.

Due to the labour-intensive nature of the study, data were collected in three different years. They were pooled in the statistical analysis as well as analysed separately. To analyse clutch-size data, the non-parametric Kruskal–Wallis ANOVA by ranks was used, as implemented in Statistica 99 edition, kernel release 5.5 (Statsoft Inc., 1999). The degrees of freedom for each comparison between plants was 1.

Results

Mean sizes of clutches for all females were 1.60 for *U. dioica*, $n = 43$ (ranging from 1.0 to 3.11 in mean for individual females; in total 660 oviposition observations) and 1.18 for *B. pubescens*, $n = 23$ (ranging from 1.0 to 1.43 in mean for individual females; in total 278 oviposition observations). The variance in clutch size was higher for *U. dioica* (0.20) than for *B. pubescens* (0.03) because of the differences in clutch size means; for this reason a non-parametric method was used in the comparison below. Clutch sizes ranged from one to nine eggs on *U. dioica* and from one to three eggs on *B. pubescens*.

In all three years, females ovipositing on the high-ranked host plant *U. dioica* laid larger clutches compared with females ovipositing on the low-ranked host plant *B. pubescens* (Fig. 1). The difference was significant when all three years were pooled: Kruskal–Wallis ANOVA by ranks, with means for females as dependent variable, all years $P \ll 0.001$ ($H = 17.03$; *Urtica* $n = 43$ females, *Betula* $n = 23$ females; 938 oviposition observations).

There were also significant differences in two out of three years when analysed separately: Kruskal–Wallis ANOVA, year 1 $P = 0.6162$ ($H = 0.251$; *Urtica* $n = 18$, *Betula* $n = 6$; 448 oviposition observations), year 2 $P = 0.0299$ ($H = 4.72$; *Urtica* $n = 7$, *Betula* $n = 3$; 182 oviposition observations), year 3 $P \ll 0.001$ ($H = 17.32$; *Urtica* $n = 18$, *Betula* $n = 14$; 308 oviposition observations).

Discussion

The results presented here show that ovipositing female comma butterflies respond to host quality by increasing clutch sizes when laying eggs on a high-quality host plant. This type of clutch-size regulation in response to host quality is predicted by theory but has seldom, if ever, been explicitly reported in insects. However, it seems likely that it could be a common phenomenon, and that more instances can be found in the literature using other terminology. Many studies report that more eggs are laid on good host plants (see, for example, Thompson, 1988; for references) but in such studies egg numbers are typically counted at the end of the day or experiment, without visual observation of discrete oviposition events, and hence actual clutch sizes cannot be determined. Leyva *et al.* (1991) found that the tephritid fly *Anastrepha ludens* lays larger egg clutches in peach than in other tested fruit, and from reported performance data peach seems to be the best host for their offspring. For some insects where quality and quantity have been partly confounded in studies (Pilson & Rausher, 1988; Damman, 1991; Freese & Zwölfer, 1996) it may be the case that they in fact also show clutch-size regulation in response to quality, in the sense of this paper, besides a response to quantity.

In one of the few studies where host quantity and host quality were unequivocally separated, Díaz-Fleischer and Aluja, 2003) actually found a negative rather than a positive response to host quality (fruit ripeness within the same species) in *A. ludens*. They suggested a number of possible explanations for their results, including that females may factor in low larval survival into optimal clutch sizes, i.e. that larger clutches in unripe fruit ensure that 'the fruit is exploited fully in the face of high mortality'. This seems unlikely to be the sole explanation, as it would mean that females when given a choice place more eggs in low-fitness environments than in high-fitness environments. The authors, however, also suggested that larger groups of larvae can accelerate the ripening process and mutually detoxify the unripe fruit. This type of explanation, including an advantage for the larvae of belonging to a large group, seems more likely on theoretical grounds. Whatever the case, the contrast between the findings reported here and those of Díaz-Fleischer and Aluja, 2003) graphically demonstrates that predictions regarding clutch-size regulation (positive, negative, or none) will differ depending on the specifics of each insect system, opening up an interesting research area. So why is positive clutch-size regulation found in the comma butterfly?

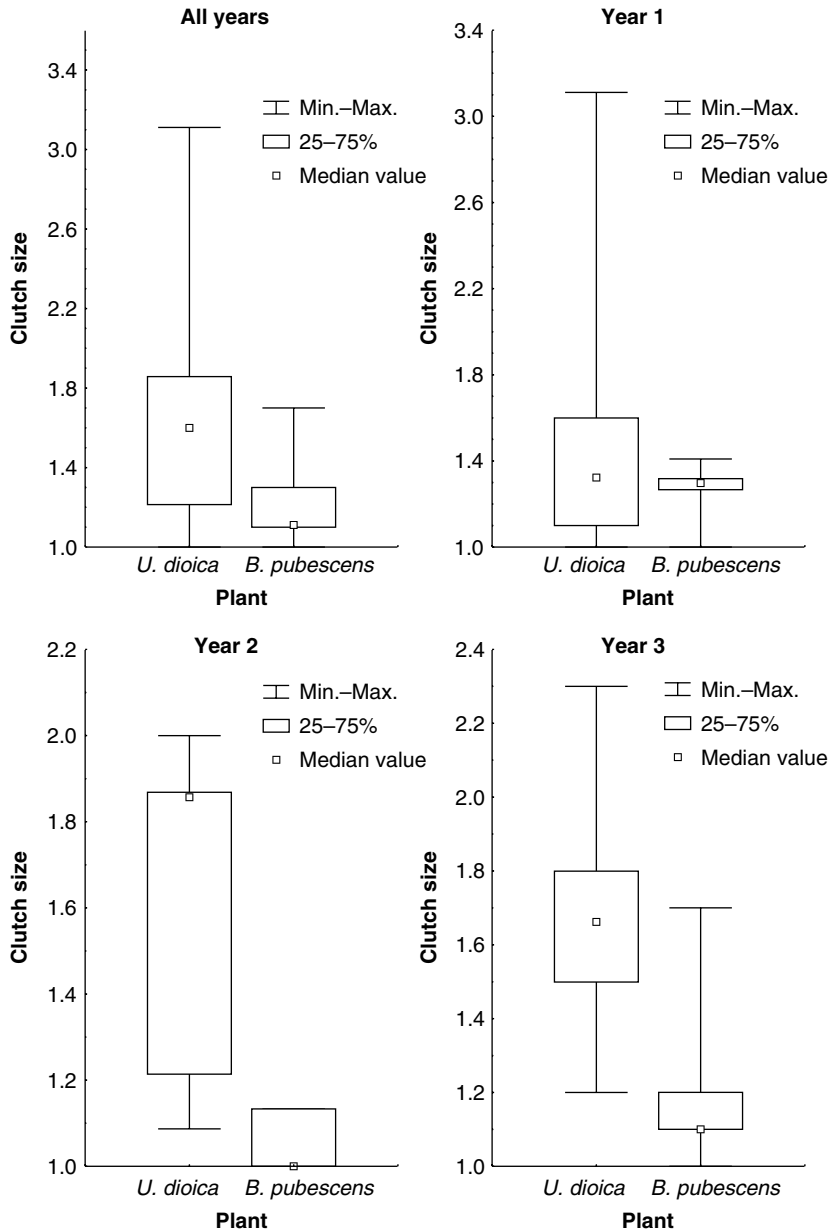


Fig. 1. Median clutch sizes on the high-quality host *Urtica dioica* and the low-quality host *Betula pubescens* for ovipositing females of *Polygona c-album*, in three different years and for all years pooled.

For species that lay large egg batches it is of vital importance for the female to choose suitable host plants with respect to both quality and quantity. The female cannot choose on quality alone because the host has to be of such a quantity that all the larvae in the clutch have the potential to reach a larval stage where survival is likely. Resource quantity is also likely to be of high importance when host plants are rare or spatially separated and larvae are unlikely to find a new host if the present one is defoliated at an early stage.

The modest clutch sizes of the comma butterfly, in combination with the growth form of its host plants, are likely to enable females to prioritise host quality over host quantity. Stinging nettles often grow in dense stands and larvae

have a high probability of finding new nettles if the original host is depleted (comma larvae are capable of searching for hosts even when newly hatched: Nylin & Janz, 1996; Nylin *et al.*, 2000). The other typical hosts of the comma butterfly are trees and bushes, which provide ample food in relation to the small clutch size.

It is well established that the hosts tested here differ greatly in quality. *Urtica dioica* supports higher survival and faster growth than *B. pubescens*. This is reflected by a higher preference for *U. dioica* in choice tests, by both females and larvae (Janz *et al.*, 1994; Nylin & Janz, 1996). It thus seems likely that *U. dioica* provides a stronger stimulus for oviposition and feeding (probably at least partly chemical), and the same stimulus could also provide a

'quality cue' for clutch-size regulation. Although possible, there is no need to invoke a separate mechanism; it can be envisaged that landing on a nettle stimulates oviposition so strongly that more than one egg is laid before the female leaves. This means that positive clutch-size regulation (as found here) may partly be seen as a side-effect of the preference hierarchy, and it could thus evolve more easily than if a separate cue was needed.

If such behaviour were not adaptive there is no reason why it could not be selected against, as shown by *A. ludens*, where females lay larger clutches on low-quality hosts (Díaz-Fleischer & Aluja, 2003). This particular example concerns variation in host quality within rather than between plant species, but there is no principal difference, because females must use some cues to determine quality within species, if they do discriminate at all. In some cases the variation in cues is evidently even stronger within than among species, as evidenced by females ranking individual plants rather than species (Singer & Lee, 2000). Swedish comma butterflies do not prefer high-quality plant individuals over low-quality individuals in experiments with stinging nettles (Janz & Nylin, 1997). For this reason, females of the Swedish population were not expected to respond to the cues of quality that distinguish individuals within species; hence, clutch-size regulation was not studied at this level.

The strategy of laying larger clutches when encountering host plants of high quality has several potential advantages. An increase in clutch size will decrease female search time. Even a slight increase in the number of eggs laid per oviposition bout will have a large positive effect. If a female, on average, oviposits two eggs instead of a single egg, then host plant search and oviposition times are cut in half. If the comma butterfly is time limited, realised fecundity can then, potentially, increase twofold. The comma butterfly is more likely to be time limited than egg limited. Weather conditions are usually very unpredictable in Sweden, especially during the early spring flight time, and there are frequently long periods of unsuitable weather conditions for female oviposition.

In addition, the risk of predation for ovipositing females is minimised. Butterflies are relatively conspicuous when flying and are therefore exposed to predators (Stamp, 1980). Flying between every single oviposition event would lead to a high degree of exposure. A decrease in search behaviour can therefore lower the risk of being predated on.

Finally, time spent foraging can be decreased. Intensive searches for suitable host plants are energetically costly and increase the amount of time a female needs to spend foraging. This will further increase the risk of predation and wastes valuable time that could instead be used for oviposition.

Conversely, laying many small clutches instead of a few large clutches allows a female to spread her eggs across many different host plants and host species. This can be viewed as a form of risk spreading that can be advantageous in a heterogeneous environment, both for eggs and

larvae. In other words, it is a way of 'not putting all the eggs in one basket' but still putting more eggs in the best basket.

In conclusion, female oviposition choices influence the fitness of their offspring (Van Nouhuys *et al.*, 2003) and a slight increase in clutch size when encountering a high quality host is likely to be a good strategy for increasing a female's fitness. This strategy increases oviposition rate, decreases search times, and associated costs while still keeping the benefits of risk spreading. If these latter benefits become less important, or the benefits of laying larger clutches become more important, due to an environmental change, the strategy of adjusting the reproductive output in response to host-plant quality could also be a first evolutionary step towards a life history where females lay large batches – on only the best hosts. Fordyce and Nice (2004) have shown geographic variation in clutch size between populations of the pipewine swallowtail butterfly, which they suggested to be a result of differences in host-plant characteristics, demonstrating the capacity for clutch sizes to evolve in response to host plants. The final step could be a specialist large-batch layer, like the small tortoiseshell *Aglais urticae* (L.) or the peacock *Inachis io* (L.), both of which are nettle specialists in the same butterfly tribe as *Polygonia* (Nylin *et al.*, 2001).

These results demonstrate the importance of considering host quality *per se* in studies of clutch-size regulation in insects. It is particularly important to avoid confounding the quality of the offspring's environment with the quantity of resources available to the offspring. Although the latter is often an important aspect of the former, this is not always the case. This distinction is also important when constructing theoretical models of clutch-size regulation in insects, as it may influence the predictions. It seems likely that the same considerations could be extended also to other animal systems where the quality of the egg-laying site is variable.

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