Ecological Entomology (2007), **32**, 235–242

Life-history, genotypic, and environmental correlates of clutch size in the Glanville fritillary butterfly

MARJO SAASTAMOINEN Department of Biological and Environmental Sciences, PO Box 65, Viikinkaari 1, FI-00014 University of Helsinki, Finland

Abstract. 1. Glanville fritillary butterfly (*Melitaea cinxia*) females lay up to 10 clutches of 50–300 eggs in their lifetime. Clutch size is an important life-history trait as larval group size affects survival throughout larval development.

2. Two experiments were carried out in a large population cage in the field to investigate the life-history and environmental correlates of clutch size.

3. Clutch size decreased with the cumulative number of eggs laid previously, increased with both female body weight and the number of days between consecutive clutches.

4. Genotypic differences among females in the glycolytic enzyme phosphoglucose isomerase had a significant influence on clutch size, partly because females of particular genotypes were able to initiate oviposition earlier in the day and thereby take advantage of the most favourable environmental conditions for oviposition.

5. Factors influencing clutch size were partly different in two summers, indicating the modulating effect of prevailing environmental conditions on reproductive performance.

Key Words. Clutch size, Glanville fritillary butterfly, oviposition, phosphoglucose isomerase.

Introduction

Clutch size is a life-history trait that has various consequences for reproductive performance, population regulation and dynamics. Causes of clutch size variation have been examined in many studies of birds (e.g. Monaghan & Nager, 1997), amphibians (e.g. Chamaille-Jammes *et al.*, 2006), and parasitic wasps (Jervis & Ferns, 2004 and references therein). In an influential paper, Lack (1947) suggested that the average clutch size in birds is determined by the number of young the parents can successfully raise under given environmental conditions. In many parasitoids, on the other hand, females adjust clutch size according to the size of the host apparently in order to maximize the size and the fitness of their offspring (Godfray & Shimada, 1999).

The majority (90–95%) of butterfly species lay their eggs singly (Stamp, 1980; Hebert, 1983), but for those species that oviposit egg clusters clutch size is an important fitness component, as increased clutch size is known to, e.g. reduce egg desiccation and often also the risk of predation or parasitism (Stamp, 1980; Kuussaari, 1998). In these species, larvae typically live gregariously at least during the early larval instars and in some cases until pupation (Fitzgerald, 1993; Kuussaari *et al.*, 2004). Larvae often do better in groups due to increased feeding facilitated by conspecifics (Denno & Benrey, 1997; Reader & Hochuli, 2003), more efficient defence against predators and parasitoids (Denno & Benrey, 1997; Reader & Hochuli, 2003), a more favourable local microclimate (Klok & Chown, 1999) and, in some species, a higher quality of protective winter web for diapause spun by many larvae (Nieminen *et al.*, 2001).

There is substantial variation in clutch size within and among individuals of the Glanville fritillary butterfly (*Melitaea cinxia* L., 1758) (Kuussaari *et al.*, 2004), which may be due to genotypic differences but also to life-history and environmental factors. Studies on other butterflies have shown that female traits such as body size (Wiklund *et al.*, 1987; Rodrigues & Moreira, 2002; Boggs & Nieminen, 2004; Jimenez-Perez & Wang, 2004), egg-laying history (Wahlberg, 1995; Boggs & Nieminen, 2004), and the number of days since the last oviposition (Pilson & Rausher, 1988; Agnew & Singer, 2000) may affect fecundity and hence also clutch size. Furthermore, environmental factors such as host plant species (Kagata & Ohgushi, 2001), food limitation (Boggs & Ross, 1993), and unfavourable weather conditions (Stamp, 1980) may also affect clutch size.

Correspondence: Marjo Saastamoinen, Department of Biological and Environmental Sciences, PO Box 65, Viikinkaari 1, FI-00014 University of Helsinki, Finland. E-mail: marjo.saastamoinen@helsinki.fi

Ambient temperature can constrain reproduction in butterflies not only due to its direct effect on egg maturation (Carroll & Quiring, 1993), but also indirectly, as low ambient temperatures decrease time available for behaviours associated with reproduction (Boggs, 2003; Fischer *et al.*, 2003). By constraining flight activity (Berwaerts *et al.*, 2001), low temperatures adversely affect mate location, search of nectar plants for feeding and search of host plants for oviposition. Flight is also influenced by the metabolic pathways that produce power for active flight, and there may be variation among individuals in traits such as the catalytic efficiency of key enzymes. Watt (1977) working on *Colias* butterflies showed that different isomorphs of the glycolytic enzyme phosphoglucose isomerase (PGI) differ in their enzyme-kinetic properties, which affect glycolytic fluxes and thereby have an effect on flight performance (Watt, 1977, 1983). Watt (1992) further demonstrated that females with certain *Pgi* genotypes have increased egg-laying rate, as these individuals can fly under a broader range of weather conditions due to increased flight performance at low temperatures. To increase body temperature for flight butterflies have also evolved behavioural adaptations and adjustments, such as basking in the sun to absorb heat (e.g. Berwaerts *et al.*, 2001) as well as morphological traits such as thicker fur on the thorax (Kingsolver, 1983a, b). Thermoregulatory behaviours affecting flight activity are critically important especially in areas where environmental conditions are often suboptimal, for instance in range margins.

The metapopulation of the Glanville fritillary butterfly in the Åland Islands, south-west Finland, is known to be polymorphic for Pgi (Saccheri *et al.*, 1998). Seven different alleles have been identified in this locus, and their frequencies have remained stable over time as indicated by two studies conducted 9 years apart (Saccheri *et al.*, 1998; Haag *et al.*, 2005). Previous studies on the Glanville fritillary have shown that certain *Pgi* genotypes, involving the allele *Pgi-f*, have elevated flight metabolic rates that are associated with increased dispersal rate (Haag *et al.*, 2005), and allelic variation among populations has even consequences for population dynamics (Hanski & Saccheri, 2006). These previous findings point to the importance of PGI in the biology of the Glanville fritillary, and they suggest that this enzyme may play a role in the reproductive performance of females through its effects on flight performance, similarly to that previously reported for *Colias* butterflies (Watt, 1992). One important difference in the reproductive biology of the Glanville fritillary and the *Colias* butterflies, however, is that the former lays eggs in clusters, whereas the latter lays single eggs. In *Colias*, females may lay 15–20 eggs per day but they must move repeatedly while laying eggs on widely scattered host plants (Watt, 1992). It is thus evident why female fecundity is highly dependent on flight activity in *Colias* butterflies, but whether a comparable mechanism affects reproductive behaviour in the Glanville fritillary is unknown.

A few studies of butterflies have examined how ovipositing females laying clusters of eggs respond to particular single factors such as unfavourable weather conditions (Stamp, 1980) and food limitation (Boggs & Ross, 1993), but very little is known about how they would respond to combinations of factors under natural conditions. Understanding the factors that affect clutch size in species with gregarious larvae is vital for developing a better understanding of their life-history ecology (Kuussaari *et al.*, 2004). Such knowledge is helpful also for the more general purpose of comparing clutch size determination in insects with that in other taxa, for which more is already known (e.g. Wilson, 1994 and references therein; Wilson & Lessells, 1994; Heimpel *et al.*, 1998). This study set out to investigate the role of life-history traits and *Pgi* genotype in the reproductive performance of the Glanville fritillary butterfly. Two experiments were conducted in a large population cage in the field in two consecutive years, allowing the assessment of prevailing environmental conditions on clutch size.

Materials and methods

The Glanville fritillary butterfly

The Glanville fritillary has a classic metapopulation in the Åland Islands in Finland, in a network of ≈ 4000 habitat patches, of which some 500 are occupied in a given year (Hanski, 1999; Nieminen *et al.*, 2004). Larvae feed on two host plant species, *Plantago lanceolata* and *Veronica spicata*. *Plantago lanceolata* occurs in all parts of Åland, whereas *V. spicata* mainly occurs in the west, north-west, and north of the archipelago (Nieminen *et al.*, 2004). *Veronica spicata* is preferred by ovipositing females in the areas where both host plants are present (Kuussaari *et al.*, 2004), even though no clear differences in larval survival have been found between the two host plant species (van Nouhuys *et al.*, 2003; Kuussaari *et al.*, 2004).

The butterfly has a univoltine life cycle in the Åland Islands. Eggs are laid in clusters and the larvae develop through five instars before the end of the summer, when they spin a compact 'winter nest', in which they diapause. The larvae become active again as soon as the snow has melted at the end of March. The larvae live gregariously until the last larval instar and pupate at the beginning of May. Adult butterflies eclose at the beginning of June with a full complement of oocytes at emergence (Boggs & Nieminen, 2004). The flight season continues until early July (Nieminen *et al.*, 2004), and females lay up to seven (Wahlberg, 1995; Kuussaari, 1998) or even 10 (this study) egg clusters during their lifetime. Adults feed on nectar, and they are capable of behavioural thermoregulation via dorsal solar basking, a behaviour that they typically exhibit in the morning prior to flight and during the day whenever necessary (pers. obs.).

Experimental set-up

Experiments were conducted in a large cage (32 × 26 × 3 m) erected upon a natural habitat patch in the Åland Islands (Hanski *et al.*, 2006). The cage was covered with a mesh preventing the butterflies from escaping but allowing natural environmental conditions (rain, sun, and wind) in. A similar apparatus has been used in studies of butterfly movement behaviour by Norberg *et al.* (2002). About 150 potted host plants were placed in the central part of the cage, which was relatively bare of vegetation compared with the margins of the cage. During the experiment carried out in 2003, only *P. lanceolata* was used as an oviposition host plant, whereas in 2004 either *P. lanceolata* or *V. spicata* was present in the cage on any given day. The margins of the cage had higher vegetation and greater density of flowering plants that provided nectar for adult butterflies.

Between the 14th and 16th April 2003, 400 fifth instar larvae were collected from 40 different local populations in the Åland Islands (Hanski *et al.*, 2006). The larvae originated from parts of the Åland Islands that are known to prefer *P. lanceolata* as a host plant species (Kuussaari *et al.*, 2000). The larvae were reared in growth chambers (L:D 12:12 h, 25 °C and 20 °C respectively) and fed with *P. lanceolata*. Approximately 24 h after pupation, individual pupae were weighed on a Scaltec SBC 33 electrobalance (sensitivity 0.1 μg, Scaltec Instruments, Heiligenstadt, Germany) and placed individually in small plastic cups, in which they were kept until eclosion. After eclosion, wing expansion and drying, the butterflies were individually numbered by permanent marker pen on the underside of the hind wing. Altogether 81 females and 113 males were released into the cage, mostly in the afternoon of the day of their eclosion.

Between the 2nd and 12th June 2004, adult butterflies instead of larvae were collected from the wild, from 25 different populations in the Åland Islands. Butterflies were sampled from both *P. lanceolata* and *V. spicata*-preferring populations (Kuussaari *et al.*, 2000). Only fresh and unworn butterflies were collected. The butterflies were exposed to temperatures of +5 °C for 5–10 min to minimise stress while they were weighed and individually numbered as described above. Marked butterflies were released into the population cage in the evening of the day they were collected.

The population cage was divided into a 8 × 8 grid, each cell measuring 4 × 2.6 m in size. Systematic censuses were conducted through the cage every second hour, from 09.00 h until 17.00 h. Censuses were not conducted during completely rainy or cloudy periods when butterflies were not active. During each census, the position of each butterfly observed was recorded. Extra caution was taken to observe mating pairs. To obtain information about egg-laying, the potted host plants were continuously monitored and the initiation of each egg-laying was recorded as well as the number of the female. Observing ovipositing females was relatively easy. In 2003 and 2004, only 32 and five of 194 and 97 ovipositions were missed, as indicated by careful search for egg clusters on the experimental plants every evening. Temperature was measured in the centre of the cage every second hour (Thermo in/out digital thermometer, ORA). The ambient temperature during egg-laying was approximated by the measurement made at the closest hour.

A measurement of butterfly mobility in the cage was obtained from a residual from the regression on the number of grid cells visited by a butterfly during its first 3 days of life against the number of observations made during the same 3 days. The mobility measure for < 4-day-old butterflies was used because it has been shown that such pre-reproductive females show different patterns of mobility among different population types (Hanski *et al.*, 2006).

Egg clutches were removed along with the leaf on which they were laid after the female had left the plant. Eggs were taken into the laboratory, where they were carefully counted with a fine paintbrush at the age of 3 days.

Additional weather data (average and maximum daily temperatures, hours of sunshine, and mm of rain) were obtained from the official Weather Service data loggers situated in Jomala and Mariehamn airport in the Åland Islands at distances of 15 and 25 km from the population cage.

Genotyping for Pgi allozyme

In 2004 female butterflies were collected from the cage when their wings were observed to be too worn for them to conduct standard flight ($n = 25$). Butterflies were flash frozen in liquid nitrogen and stored at –80 °C until analysis. Electrophoresis analysis was carried out by C. Haag according to the protocol given by Hebert and Beaton (1993) to assess allelic variation in Pgi (EC number 5.3.1.9; for more details see Haag *et al.*, 2005).

Data analysis

The effect of *Pgi* genotype on the timing of the initiation of oviposition and on clutch size was studied in the experiment conducted in 2004. Based on the results of Haag *et al.* (2005), females were divided into two classes, those having the *Pgi-f* allele (one or two copies) and those not having it. ANCOVA was used to test whether females with *Pgi-f* allele laid larger clutches than females without *Pgi-f* with temperature at the initiation of egg-laying as a covariate. A second analysis was performed to test whether there was a difference between *Pgi-f* and *non-Pgi-f* females in the time of the day of oviposition. Days during which only one of the two female types had laid eggs were omitted from this analysis. To standardise data for the remaining 11 days on the time of the day when egg-laying was initiated, the daily average was subtracted from the value for each clutch and the difference was divided by the daily standard deviation. The standardised values for the Pgi classes were compared with *t*-test.

Linear Mixed Effects Model of repeated measures with individual as a random factor (PROC MIXED of SAS version 8.02; SAS Institute, 1999) was used to examine which other female traits apart from the *Pgi* genotype affected clutch size. Many variables were initially included in the models (clutch number, butterfly mobility, host plant species), and model selection was done by backward elimination of non-significant factors. Second-order interactions were not kept in the final model as they were all non-significant and did not increase the model fit (AIC-value). In 2004, a categorical variable reflecting the host plant preference of the ovipositing female was added to the initial model as this year females were collected from both *P. lanceolata* and *V. spicata* preferring areas. This variable had the value of 1 if the female oviposited on the host plant that it was considered to prefer (based on geographical variation in preference; Kuussaari *et al.*, 2000), otherwise the variable had the value of 0. Correlations between the explanatory variables were checked and if they were strong only one of the variables, the biologically more plausible one, was used in the final model.

Results

Female life-history traits and clutch size

One hundred and ninety-four and 97 egg clutches were laid by 65 and 39 females respectively, in 2003 and 2004. The average clutch size was substantially smaller in 2003 (130) than in 2004 (191, $t_{(289)} = 8.4$, $P < 0.0001$), whereas the average number of clutches per female was about the same, about three (Table 1). The average length of time between ovipositions was much shorter (2.7 days) in 2003 than in 2004 (4.4 days, $t_{(203)} = -5.68$, $P = 0.05$; Table 1).

Clutch size increased with female weight in 2004 (Table 2 and Fig. 1a) but not in 2003 (weight measured as pupal weight; $F_{1,84} = 0.0$, $P = 0.95$). The cumulative number of eggs that the female had laid previously was negatively correlated with the current clutch size in both years (Table 2; Fig. 1b shows the effect in 2004). In 2003, the longer the time interval between two consecutive ovipositions the larger the latter clutch (Table 2, Fig. 1c), but in 2004 there was no such effect ($F_{1,30} = 0.55$, $P = 0.47$).

In 2003, 28% of the females mated more than once. Clutches of females that mated more than once were smaller than clutches of females that mated only once (Table 2). There was no difference in the clutch sizes of polyandrous females when clutches prior to the second mating were compared with those laid after the second mating ($t_{(71)} = 1.62$, $P = 0.12$). The effect of the number of matings was not analysed in 2004 due to the collection procedure of the butterflies (many females may have mated before collection).

Time of the day at the initiation of egg laying was significantly associated with clutch size in both years (Table 2): clutches laid earlier in the day were bigger (Fig. 1d shows the effect in 2004). Finally, clutch number (in both 2003 and 2004), host plant species, and host plant preference (only in 2004) did not affect clutch size in either of the experiments. The *P*-values for clutch number and mobility were 0.203 and 0.348 respectively, in 2003, and for clutch number, mobility, host plant species and host plant preference 0.103, 0.770, 0.302, and 0.638 respectively, in 2004.

Table 1. Summary of the data for 2003 and 2004. Average and standard deviation are given.

	2003	2004
No. of females	65	39
No. of populations*	32	26
No. of clutches	194	97
Clutch size	130 ± 61.6	191 ± 60.0
No. of clutches laid/female	3.0 ± 2.1	2.6 ± 1.5
Time between ovipositions (days)	2.7 ± 1.9	4.4 ± 2.6
Weight (mg)	133 ± 12.7†	130 ± 17.6

*From which the butterflies originated (see Materials and methods).
†Pupal weight transformed to adult weight for comparison between the years.

Table 2. Models of clutch size in the Glanville fritillary in 2003 and 2004. Individual female was used as a random factor. num, numerator; den, denominator.

Factor	num d.f.	den d.f.	*F*	*P*
2003				
Number of eggs laid previously	1	85	10.16	0.002
Days since the last clutch was laid	1	85	18.47	< 0.0001
Time at the initiation of oviposition	1	85	9.91	0.0023
Number of matings	1	85	5.7	0.019
2004				
Number of eggs laid previously	1	57	12.08	0.001
Weight	1	57	5.97	0.0177
Time at the initiation of oviposition	1	57	12.55	0.0008

Pgi genotype, daily timing of oviposition and clutch size

Females with the *Pgi-f* allele started oviposition earlier during the day than females without this allele (*t*-test on standardised values; $t_{(70)} = -2.96$, $P = 0.0041$, Fig. 2a). There was a significant negative correlation between the time of the day and temperature ($R^2 = 0.29$, $P < 0.001$), and thereby temperature was highest during the early hours of the afternoon when the *Pgi-f* females tended to start oviposition. Females with the *Pgi-f* allele also laid larger clutches than females without this allele (ANCOVA; *Pgi*-type, $F_{1,73} = 9.02$, $P = 0.0037$; temperature, $F_{1,73} = 17.39$, $P < 0.0001$, Fig. 2b).

Weather conditions

Data obtained from the Weather Service data loggers located 15 and 25 km away from the population cage showed a substantial difference between the two years. There were no significant differences in the hours of sunshine or the amount of precipitation per day, nor in the maximal temperatures, but the mean daily temperature was significantly lower in 2004 than in 2003 (ANOVA $F_{1,54} = 4.09$, $P < 0.05$). Temperature data from the cage indicated a significant difference in the maximal daily temperature between the years (ANCOVA; year, $F_{1,36} = 10.34$, $P < 0.003$; date, $F_{1,36} = 3.5$, $P = 0.07$, Fig. 3). Furthermore, interaction between year and date showed that in 2003 temperature increased towards the end of the experiment, whereas in 2004 the opposite was the case (ANCOVA; year × date, $F_{1,36} = 15.0$, $P = 0.004$, Fig. 3). These results will be used in the interpretation of the results below.

Discussion

Several factors affected clutch size in the Glanville fritillary butterfly. Clutch size decreased with increasing number of eggs laid previously in both experiments. The number of eggs laid previously is highly correlated with female age. In many insect

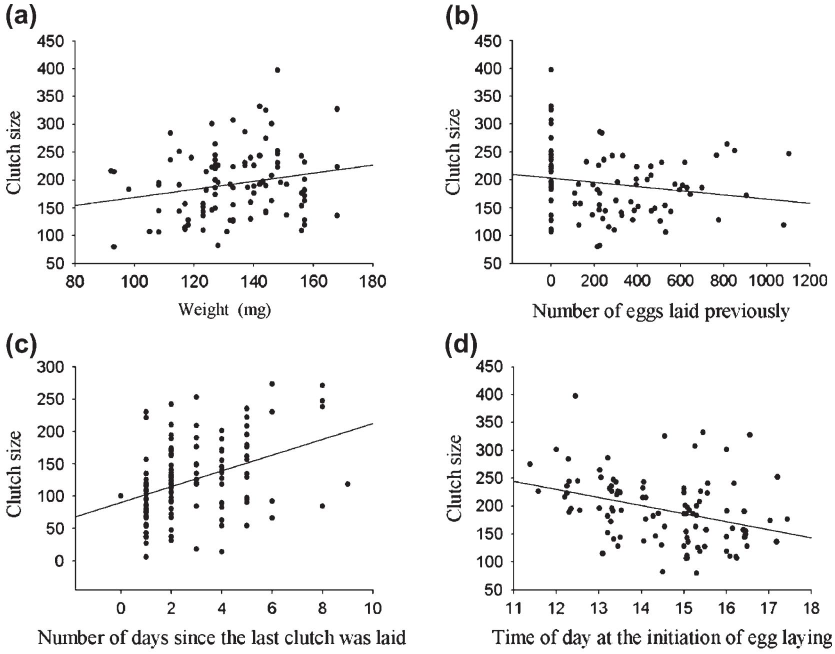

Fig. 1. Clutch size in relation to body weight (A), number of eggs laid previously (B), number of days since the last clutch was laid (C), and the time of the day at the initiation of egg laying (D). For statistics see Table 2. (C) Results are from the 2003 experiment, the rest from the 2004 experiment.

species, including Lepidoptera, both egg size and clutch size decrease with female age (e.g. Karlsson & Wiklund, 1984; Braby & Jones, 1995; Boggs, 1997b; Yanagi & Miyatake, 2002; Javois & Tammaru, 2004; O'Brien *et al.*, 2004), possibly due to resource depletion, which increases under stressful conditions (Boggs, 1997a, b; Yanagi & Miyatake, 2002). According to this hypothesis, females start to run out of resources available for egg production as they become older and, in some cases, may even absorb some of the oocytes and reallocate the nutrients. The amount of carbon in eggs that derives from larval resources, and the point in the adult life at which the shift from using larval to adult resources occurs, is not known for the Glanville fritillary.

Running out of nutrients received from males in nuptial gifts (Wedell *et al.*, 2002) is not likely to be important in the Glanville fritillary. First, even though in the present study females re-mated relatively frequently (28% in 2003), previous observations in the wild have indicated that Glanville fritillary females only rarely mate more than once (Kuussaari, 1998). The relatively high incidence of multiple matings in the cage may reflect the density of butterflies, which is generally lower in natural populations than in the cage. In 2003 polyandrous females laid smaller rather than larger clutches than monoandrous females. This suggests that females that mate multiply are not able to compensate, not at least fully, for possible shortage of nutrients or sperm by mating for the second time. Polyandrous females laid smaller clutches even prior to their second mating. Sarhan and Kokko (2007) suggest that polyandrous females lay smaller clutches but more frequently than monoandrous females as a form of risk spreading, as their reproductive success may depend on the genotypes of the different mates. Finally, greater allocation of resources to earlier than later clutches may also be related to uncertainty of surviving until later opportunities for oviposition (Javois & Tammaru, 2004).

The interval between consecutive ovipositions under favourable conditions is generally 2 days in the Glanville fritillary,

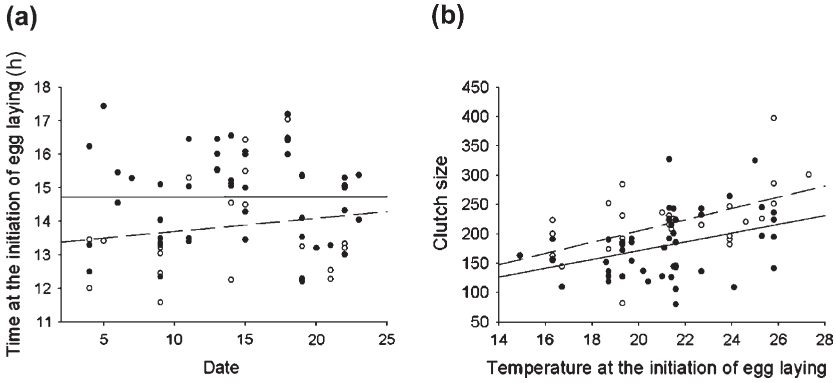

Fig. 2. The relationships between the time of the initiation of egg-laying and date (A) and clutch size and temperature at the initiation of egg-laying (B). Open and closed symbols indicate females with and without the *Pgi-f* allele respectively.

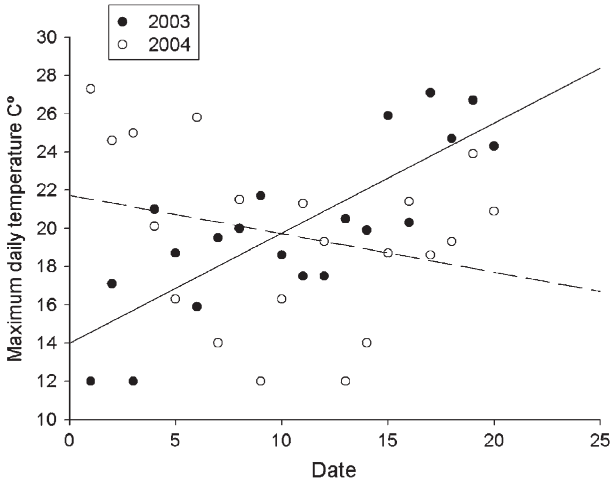

Fig. 3. The maximum daily temperatures (°C) during the experiments in June 2003 and 2004 (temperature measured in the centre of the cage with Thermo in/out digital thermometer).

although occasionally oviposition occurs in consecutive days (Boggs & Nieminen, 2004). Egg load is presumed to increase with time since the last oviposition (Agnew & Singer, 2000; Singer, 2004) and increased egg load possibly causes butterflies to lay larger clutches (Pilson & Rausher, 1988). In the present study, clutch size increased with increased interval between ovipositions in 2003, but there was no such relationship in 2004. Clutch sizes in general, however, were larger in 2004 when the clutches were laid at longer intervals than in 2003. Similar observations have been made in other Lepidoptera (Stamp, 1980). Longer intervals between consecutive ovipositions in 2004 than in 2003 may be due to prevailing weather conditions, which were less favourable in 2004 (Fig. 3a,b). Provided that temperature exceeds a threshold value egg maturation is still likely to occur, though at a lower rate, under poor weather conditions. For example, in the tortricid moth *Zeiraphera canadensis* the rate of egg maturation increases linearly with temperature, and eggs are matured at low rate at the low temperatures of 10–15 °C (Carroll & Quiring, 1993). In 2004, the daily temperatures were close to 15 °C and the average number of sunny hours per day greater than 10. Thus, even though in 2004 females were not able to lay eggs as often as in 2003 they were able to compensate for this by laying larger clutches.

Increased body size has a positive effect on individual egg size (e.g. Wiklund *et al.*, 1987; Garcia-Barros, 1994, 2000) and on egg numbers (Wiklund *et al.*, 1987; Rodrigues & Moreira, 2002; Boggs & Nieminen, 2004; Jimenez-Perez & Wang, 2004) in many Lepidoptera. The most common reason for reduced adult body size in butterflies is reduced larval food quality and/or quantity (Awmack & Leather, 2002; Boggs, 2003; Boggs & Freeman, 2005). In the present study large females laid larger egg clutches than small females only in 2004. There was no difference in body size between the two years, suggesting that the quality and the quantity of larval resources was similar in the two years. Adult butterflies are likely to be able to compensate for small body size by feeding (O'Brien *et al.*, 2004). In the present experiments, butterflies moved freely and were able to feed on abundant nectar flowers in the cage, but the less favourable weather conditions in 2004 may have reduced adult feeding. In short, small butterflies in 2004 may have been unable to compensate for their small body reserves by adult feeding, which may have reduced their fecundity.

Pgi genotypes, daily timing of oviposition, and clutch size

Previous studies on *Colias* butterflies have shown that individuals have dissimilar flight activity periods due to different genotypes of the glycolytic enzyme PGI (Watt, 1983; Watt & Dean, 2000). Watt *et al.* (1983) showed that particular heterozygotes for the *Pgi* locus were kinetically most effective at low temperatures, and they were thus able to begin their flight earlier in the day than the other genotypes. Haag *et al.* (2005) found that *Pgi* polymorphism is related to variation in flight metabolic performance in the Glanville fritillary, such that those individuals that have the *Pgi-f* allele have higher flight metabolic rate than individuals lacking this allele.

Results from the present study are consistent with those on *Colias* butterflies; *Pgi* affected significantly the reproductive behaviour of the Glanville fritillary. Females with the *Pgi-f* allele started to oviposit earlier in the day than females without this allele, probably due to the ability of females with *Pgi-f* to fly at lower temperatures in the morning. Prior to oviposition butterflies often feed (pers. obs.), and females that can do this at lower temperatures in the morning could thereby initiate oviposition during the early hours of the afternoon when temperatures are usually highest.

Females with *Pgi-f* also laid larger clutches than those without *Pgi-f*. As the time of the day when oviposition was started was correlated with clutch size in both years, it is likely that the increased clutch size of the *Pgi-f* females is at least partly due to the timing of their oviposition. One possible reason why females laid larger clutches earlier in the day is higher ambient temperatures. A strong negative correlation between temperature and the time of the day in the afternoon was apparent in both years. Prevailing weather conditions can greatly affect oviposition, as most butterflies are inactive under overcast, windy, and rainy weather (Stamp, 1980) and hence unable to search for nectar and oviposition plants (Kingsolver, 1983a, b). A few studies have looked at the role of temperature on egg-laying more directly. Wiklund *et al.* (1987) showed that satyrid species living in low temperature environments have reduced the maximum number of eggs laid per day compared with species of satyrids adapted to warm habitats. Furthermore, Karlsson and Wiklund (2005) showed that lifetime egg production of four satyrid species is greatly affected by temperature, with all species having an optimal temperature in which they lay the largest number of eggs. Other factors such as humidity, solar radiation, and so forth, may also be causally related to increased clutch size at higher temperatures.

Favourable conditions at the time of oviposition is not, however, the only reason for the larger clutches laid by females with the *Pgi-f* allele, as the effect of temperature was accounted for in the final model (Fig. 1b). *Pgi-f* females have high (flight) metabolic performance (Haag *et al.*, 2005), which may affect their rate of oviposition or egg maturation and thus increase clutch size. The present study cannot refute the possibility of some other inherent quality differences, for example due to differential larval development rate, between females with and without *Pgi-f* allele.

Understanding the factors that influence clutch size is particularly important in species in which offspring survival is dependent on larval group size. This is the case in the Glanville fritillary, in which the survival of all larval stages is affected by group size, and no negative effects of group size have been observed at any naturally occurring group sizes (Kuussaari *et al.*, 2004). The present study was undertaken in a population cage under practically natural conditions, which did not allow controlling of the environmental conditions, but at the same time these results, replicated for two summers, do not require any extrapolation to account for conditions encountered by females living in their natural environment. The results demonstrate that many factors influence clutch size in the Glanville fritillary, including allelic variation in a key metabolic enzyme (PGI) that allows females with certain genotypes to initiate their oviposition earlier in the day than other females. These results illustrate how a range of phenotypic and genotypic traits combine with environmental factors to determine clutch size and thereby influence individual fitness and potentially affect population dynamics.

Acknowledgements

Special thanks to Ilkka Hanski, Christoph Haag, Jim Marden, and the cage teams 2003 and 2004 for their help with the experiments. Ilkka Hanski, Saskya van Nouhuys, Christopher West Wheat and two anonymous referees are acknowledged for their comments on the manuscript. Regional Council of the Åland Islands is thanked for permitting the use of their land for the experiments. Funding for this project was provided by the Academy of Finland (Finnish Centre of Excellence Programme, 2000–05, grant number 20286). The experiments described in this study comply with the current laws of Finland.

References

Agnew, K. & Singer, M.C. (2000) Does fecundity constrain the evolution of insect diet? *Oikos*, **88**, 533–538.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.

Berwaerts, K., Van Dyck, H., Vints, E. & Matthysen, E. (2001) Effect of manipulated wing characteristics and basking posture on thermal properties of the butterfly *Pararge aegeria* (L.). *Journal of Zoology*, **255**, 261–267.

Boggs, C.L. (1997a) Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology*, **78**, 192–202.

Boggs, C.L. (1997b) Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology*, **78**, 181–191.

Boggs, C.L. (2003) Environmental variation, life histories, and allocation. *Butterflies: Ecology and Evolution Taking Flight* (ed. by C. L. Boggs, W. B. Watt and P. R. Ehrlich), pp. 185–206. The University of Chicago Press, Chicago.

Boggs, C.L. & Freeman, K.D. (2005) Larval food limitation in butterflies: effects of adult resource allocation and fitness. *Oecologia*, **144**, 353–361.

Boggs, C.L. & Nieminen, M. (2004) Checkerspot reproductive biology. *On the Wings of the Checkerspots: a Model System for Population Biology* (ed. by P. R. Ehrlich and I. Hanski), pp. 92–111. Oxford University Press, Oxford.

Boggs, C.L. & Ross, C. (1993) Reproductive variation during adult nutrient limitation in *Speyeria mormonia*. *Ecology*, **43**, 152–161.

Braby, M.F. & Jones, R.E. (1995) Reproductive patterns and resource allocation in tropical butterflies: influence of adult diet and seasonal phenotype on fecundity, longevity and egg size. *Oikos*, **72**, 189–204.

Carroll, A.L. & Quiring, D.T. (1993) Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis*. *Oecologia*, **93**, 233–241.

Chamaille-Jammes, S., Massot, M., Aragon, P. & Clobert, J. (2006) Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biology*, **12**, 392–402.

Denno, R. & Benrey, B. (1997) Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology*, **22**, 133–141.

Fischer, K., Eenhoorn, E., Bot, A.N.M., Brakefield, P.M. & Zwaan, B.J. (2003) Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. *Proceedings of the Royal Society of London Series B Biological Sciences*, **1528**, 2051–56.

Fitzgerald, T.D. (1993) Sociality in caterpillars. *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (ed. by N. E. Stamp and T. M. Casey), pp. 372–403. Chapman & Hall, New York.

Garcia-Barros, E. (1994) Egg size variation in European Satyrine butterflies (Nymphalidae, Satyrinae). *Biological Journal of the Linnean Society*, **51**, 309–324.

Garcia-Barros, E. (2000) Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society*, **70**, 251–284.

Godfray, H.C.J. & Shimada, M. (1999) Parasitoids: a model system to answer questions in behavioral, evolutionary and population ecology. *Researches on Population Ecology*, **41**, 3–10.

Haag, C.R., Saastamoinen, M., Marden, J.H. & Hanski, I. (2005) A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proceedings of the Royal Society of London Series B Biological Sciences*, **272**, 2449–2456.

Hanski, I. (1999) *Metapopulation Ecology*. Oxford University Press Inc., New York.

Hanski, I., Saastamoinen, M. & Ovaskainen, O. (2006) Dispersal-related life history trade-offs in a butterfly metapopulation. *Journal of Animal Ecology*, **75**, 91–100.

Hanski, I. & Saccheri, I. (2006) Molecular level variation affects population growth in a butterfly metapopulation, PLoS Biology, **4**, 0719–0726.

Hebert, P.D.N. (1983) Egg dispersal patterns and adult feeding behaviour in the lepidoptera. *Canadian Entomology*, **115**, 1477–1481.

Hebert, P.D.N. & Beaton, M.J. (1993) *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis: a Practical Handbook*. Helena Laboratories, Beaumont, Texas.

Heimpel, G.E., Mangel, M. & Rosenheim, J.A. (1998) Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. *The American Naturalist*, **152**, 273–289.

Javois, J. & Tammaru, T. (2004) Reproductive decisions are sensitive to cues of life expectancy: the case of a moth. *Animal Behaviour*, **68**, 249–255.

Jervis, M.A. & Ferns, P.N. (2004) The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. *Oikos*, **107**, 449–460.

Jimenez-Perez, A. & Wang, Q. (2004) Effect of body weight on reproductive performance in *Cnephasia jactatana* (Lepidoptera: Tortricidae). *Journal of Insect Behavior*, **17**, 511–522.

Kagata, H. & Ohgushi, T. (2001) Clutch size adjustment of a leafmining moth (*Lyonetiidae: Lepidoptera*) in response to resource availability. *Annals of the Entomological Society of America*, **95**, 213–217.

Karlsson, B. & Wiklund, C. (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the Wall Brown butterfly *Lasiommata megera*. *Oikos*, **43**, 376–385.

Karlsson, B. & Wiklund, C. (2005) Butterfly life history and temperature adaptation; dry open habitats select for increased fecundity and longevity. *Journal of Animal Ecology*, **74**, 99–104.

Kingsolver, J.G. (1983a) Ecological significance of flight activity in *Colias* butterflies: implications for reproductive strategy. *Ecology*, **64**, 546–551.

Kingsolver, J.G. (1983b) Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. *Ecology*, **64**, 534–545.

Klok, C.J. & Chown, S.L. (1999) Assessing the benefits of aggregation: thermal biology and water relations of anomalous emperor moth caterpillars. *Functional Ecology*, **13**, 417–427.

Kuussaari, M. (1998) *Biology of the Glanville fritillary butterfly* (*Melitaea cinxia*). PhD thesis, University of Helsinki, Finland.

Kuussaari, M., Singer, M. & Hanski, I. (2000) Local specialization and landscape-level influence on host use in a herbivorous insect. *Ecology*, **81**, 2177–2187.

Kuussaari, M., van Nouhuys, S., Hellmann, J. & Singer, M. (2004) Larval biology. *On the Wings of Checkerspots: a Model System for Population Biology* (ed. by P. R. Ehrlich and I. Hanski), pp. 138–160. Oxford University Press, Oxford.

Lack, D. (1947) The significance of clutch size. *Ibis*, **89**, 302–352.

Monaghan, P. & Nager, R.G. (1997) Why don't birds lay more eggs? *Trends in Ecology and Evolution*, **12**, 270–274.

Nieminen, M., Singer, M., Fortelius, W., Schöps, K. & Hanski, I. (2001) Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *The American Naturalist*, **157**, 237–44.

Nieminen, M., Siljander, M. & Hanski, I. (2004) Structure and dynamics of *Melitaea cinxia* metapopulations. *On the Wings of Checkerspot: a Model System for Population Biology* (ed. by P. R. Ehrlich and I. Hanski), pp. 63–91. Oxford University Press, Oxford.

Norberg, U., Enfjäll, K. & Leimar, O. (2002) Habitat exploration in butterflies – an outdoor cage experiment. *Evolutionary Ecology*, **16**, 1–14.

van Nouhuys, S., Singer, M. & Nieminen, M. (2003) Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species. *Ecological Entomology*, **28**, 193–202.

O'Brien, D.M., Boggs, C.L. & Fogel, M.L. (2004) Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. *Oikos*, **105**, 279–291.

Pilson, D. & Rausher, M.D. (1988) Clutch size adjustment by a swallowtail butterfly. *Nature*, **333**, 361–363.

Reader, T. & Hochuli, D.F. (2003) Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation and microclimate. *Ecological Entomology*, **28**, 729–737.

Rodrigues, D. & Moreira, G.R.P. (2002) Geographical variation in larval host-plant use by *Heliconius erato* (Lepidoptera: Nymphalidae) and consequences for adult life history. *Brazilian Journal of Biology*, **62**, 321–332.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski. I. (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Sarhan, A. & Kokko, H. (2007) Multiple mating in the Glanville Fritillary butterfly: a case of within-generation bet-hedging? *Evolution*, in press.

SAS Institute (1999) *SAS/STAT Software User's Guide*, Release 8.00. SAS Institute Inc., Cary, North Carolina.

Singer, M. (2004) Measurements, correlates, and importance of oviposition preference in the life of checkerspots. *On the Wings of the Checkerspots: a Model System for Population Biology* (ed. by P. R. Ehrlich and I. Hanski), pp. 112–137. Oxford University Press, Oxford.

Stamp, N.E. (1980) Egg deposition patterns in butterflies: Why do some species cluster their eggs rather than deposit them singly? *American Naturalist*, **115**, 367–380.

Wahlberg, N. (1995) *One day in the life of a butterfly: A study of the biology of the Glanville fritillary Melitaea cinxia*. MSc thesis, University of Helsinki, Finland.

Watt, A.D. (1977) Adaptations at special loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: biochemical and population aspect. *Genetica*, **87**, 177–194.

Watt, W.B. (1983) Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias pgi* polymorphism. *Genetics*, **103**, 691–724.

Watt, W.B. (1992) Eggs, enzymes, and evolution: Natural genetic variants change insect fecundity. *Proceedings of the National Academy of Sciences, USA*, **89**, 10608–10612.

Watt, W.B. & Dean, A.M. (2000) Molecular-functional studies of adaptive genetic variation in prokaryotes and eukaryotes. *Annual Review of Genetics*, **34**, 593–622.

Watt, W.B., Cassin, R.C. & Swan, M.S. (1983) Adaptation at specific loci. III. Field behaviour and survivorship differences among *Colias pgi* genotypes are predictable from in vitro biochemistry. *Genetics*, **103**, 725–739.

Wedell, N., Gage, M.J.G. & Parker, G.A. (2002) Sperm competition, male prudence and sperm-limited females. *Trends in Ecology and Evolution*, **17**, 313–320.

Wiklund, C., Karlsson, B. & Forsberg, J. (1987) Adaptative versus constraint explanations for egg-to-body size relationships in two butterflies families. *American Naturalist*, **130**, 828–838.

Wilson, K. (1994) Evolution of clutch size in insects. II. A test of static optimality models using the beetle *Callosbruchus maculatus* (Coleoptera: Bruhidae). *Journal of Evolutionary Biology*, **7**, 365–386.

Wilson, K. & Lessells, C.M. (1994) Evolution of clutch size in insects. I. A review of static optimality models. *Journal of Evolutionary Biology*, **7**, 339–363.

Yanagi, S.I. & Miyatake, T. (2002) Effects of maternal age on reproductive traits and fitness components of the offspring in the bruchid beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Physiological Entomology*, **27**, 261–266.

Accepted 27 October 2006